MEIC Evaluation of Acute Systemic Toxicity

Part VI. The Prediction of Human Toxicity by Rodent LD50 Values and Results From 61 *In Vitro* Methods

Björn Ekwall,[1] Frank A. Barile,[2] Argelia Castano,[3] Cecilia Clemedson,[1] Richard H. Clothier,[4] Paul Dierickx,[5] Barbro Ekwall,[1] Margherita Ferro,[6] Geirid Fiskesjö,[7] Lourdes Garza-Ocañas,[8] Maria José Gómez-Lechón,[9] Michael Gülden,[10] Tony Hall,[11] Boris Isomaa,[12] Anne Kahru,[13] Gustaw Kerszman,[14] Udo Kristen,[15] Manabu Kunimoto,[16] Sirpa Kärenlampi,[17] Lillemor Lewan,[18] Anatoly Loukianov,[19] Tadao Ohno,[20] Guido Persoone,[21] Lennart Romert,[22] Thomas W. Sawyer,[23] Ravi Shrivastava,[24] Helmut Segner,[25] Annalaura Stammati,[26] Noriho Tanaka,[27] Matteo Valentino,[28] Erik Walum[22] and Flavia Zucco[29]

CTLU, Pavals, När, 620 13 Stånga, Sweden[30]

Summary — The Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) programme was set up to evaluate the relevance for human acute toxicity of *in vitro* cytotoxicity tests. At the end of the project in 1996, 29 laboratories had tested all 50 reference chemicals in 61 cytotoxicity assays. Five previous articles have presented the *in vitro* data and the human database to be used in the evaluation. This article presents three important parts of the final evaluation: a) a comparison of rat and mouse oral LD50 with human acute lethal doses for all 50 chemicals; b) a display of the correlations between IC50 (concentration causing 50% inhibition) values from all 61 assays and three independent sets of human acute lethal blood concentrations, i.e. clinical lethal concentrations, forensic lethal concentrations, and peak concentrations; and c) a series of comparisons between average IC50 values from ten human cell line 24-hour assays and human lethal blood concentrations. In the latter comparisons, results from correlations were linked with known human toxicity data for the chemicals, to provide an understanding of correlative results. This correlative/mechanistic approach had the double purpose of assessing the relevance of the *in vitro* cytotoxicities, and of testing a series of hypotheses connected with the basal cytotoxicity concept. The results of the studies were as follows. Rat LD50 predictions of human lethal dosage were only relatively good (R^2 = 0.61), while mouse LD50s gave a somewhat better prediction (R^2 = 0.65). Comparisons performed between IC50 values from the 61 assays and the human lethal peak concentrations demonstrated that human cell line tests gave the best average results (R^2 = 0.64), while mammalian and fish cell tests correlated less well (R^2 = 0.52–0.58), followed by non-fish ecotoxicological tests (R^2 = 0.36). Most of the 61 assays underpredicted human toxicity for digoxin, malathion, carbon tetrachloride and atropine sulphate. In the correlative/mechanistic study, the 50 chemicals were first separated into three groups: A = fast-acting chemicals with a restricted passage across the blood–brain barrier; B = slow-acting chemicals with a restricted passage across the blood–brain barrier; and C = chemicals which cross the blood–brain barrier freely, while inducing a non-specific excitation/depression of the central nervous system (CNS). The IC50 values for chemicals in group C were divided by a factor of ten to compensate for a hypothetical extra vulnerability of the CNS to cytotoxicity. Finally, the average human cell line IC50 values (24-hour IC50 for groups A and C, and after 48-hour for group B) were compared with relevant human lethal blood concentrations (peak concentrations for groups A and C, and 48-hour concentrations for group B). As a result, *in vitro* toxicity and *in vivo* toxicity correlated very well for all groups (R^2 = 0.98, 0.82 and 0.85, respectively). No clear overprediction of human toxicity was made by the human cell tests. The human cell line tests underpredicted human toxicity for only four of the 50 chemicals. These outlier chemicals were digoxin, malathion, nicotine and atropine sulphate, all of which have a

[30] *Individual addresses are given after the references.*

lethal action in man through interaction with specific target sites not usually found in cell lines. Potassium cyanide has a cellular human lethal action which cannot be measured by standard anaerobic cell lines. The good prediction of the human lethal whole-blood concentration of this chemical was not conclusive, i.e. was probably a "false good correlation". Another two chemicals in group C resulted in "false good correlations", i.e. paracetamol and paraquat. The comparisons thus indicated that human cell line cytotoxicities are relevant for the human acute lethal action for 43 of the 50 chemicals. The results strongly support the basal cytotoxicity concept, and further point to the non-specific CNS depression being the obligatory reaction of humans to cytotoxic concentrations of chemicals, provided that the chemicals are able to pass the blood–brain barrier.

Key words: acute poisoning, alternatives, basal cytotoxicity, clinical toxicology, cytotoxicity, evaluation, forensic medicine, human toxicity, lethal concentrations, lethal dose, toxicity, toxicology, validation.

Introduction

The Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) programme is an international collaborative study which aims to evaluate the relevance of *in vitro* toxicity assays for human acute toxicity (1–4). At the end of the 7-year programme in the summer of 1996, 29 laboratories had tested all 50 reference chemicals in 61 assays. Results had also been submitted from testing of the first 30 chemicals with 29 further assays and testing of the last 20 chemicals with six further assays. Four previous papers in this series presented all the *in vitro* toxicity results, including descriptions of the methods, as well as comprehensive comparative cytotoxicity analyses (5–8). A fifth paper presented all the animal and human toxicity data which will be used as the "best available standard" for the *in vitro/in vivo* comparisons in the evaluation (9).

The remaining papers of this series will present the methods, results and conclusions of the final MEIC evaluation. This paper will present an overview evaluation with the use of relatively simple methods, covering the most urgent aims of the programme. Four more papers are planned, which will supplement the present article with more-sophisticated in-depth analyses of specific questions.

One of the key principles of MEIC is to try to perform a "predetermined" evaluation, i.e. to define the reference data, methods and goals of the evaluation at the start of the programme and then strictly adhere to them (1). This approach has also included the initial performance of a "prototype" evaluation with a limited data set, to demonstrate the main features (2). The rationale for the predetermined approach is to avoid conclusions from a plethora of potential *ad hoc* reference data or methods, as a means of making the results more convincing. To be able to define the specific aims of this and forthcoming papers, the "predetermined" methods and goals of the total evaluation process (1, 2, 4) will be reiterated.

The main method used to evaluate the relevance of *in vitro* test results is a correlative/mechanistic approach. This approach first compares analogous *in vitro* and *in vivo* data by univariate linear regression analysis. The correlative results are then linked to the known effects and mechanisms of the chemicals, which may explain some correlations and outlier predictions (1). The correlative comparisons include modelling of human toxicity data by combining *in vitro* toxicity and kinetic data. As a first stage, a relatively simple model of human lethal blood concentrations is made and adjusted to test certain hypotheses (1, 2, 4). An unbiased soft multivariate modelling then follows, notably for use in battery optimisation (1, 2). As a second stage, a more-complicated modelling of human toxic dosage is performed (1, 4). All modelling and the mechanistic interpretation of predictions are based only on the originally compiled reference data (9), even if new data have been found which might improve correlations or better explain events.

Some of the aims of MEIC, as delineated in the initial articles, had to be abandoned, such as the evaluation of local irritancy and teratogenicity and annual publication of evaluation results. However, most of the goals set for the final evaluation of acute systemic toxicity are still valid (1–4). These include:

1. an evaluation of the relevance of rodent LD50 values for human acute lethal dosage, to provide a basis for comparisons of the predictivity of animal and *in vitro* tests (1, 2);

2. an evaluation of the results from each separate method, as a service to participating laboratories, including a similar evaluation of average results from each group of the same type of tests (1);
3. in order to improve understanding of chemical toxicity, a comprehensive evaluation of the relevance of the best tests. Modelling of human toxic concentrations (Stage I modelling) will probably suffice for this purpose (1, 4). This evaluation includes the ultimate testing of a series of hypotheses on basal cytotoxicity (10, 11), such as: a) the common occurrence of basal cytotoxicity; b) the necessity of considering passage across the blood–brain barrier, as well as the importance of brain functions for the short-term vitality of the body; (c) the probably rare occurrence of false positive *in vitro* predictions; and d) the frequent explanation of target organ toxicity as the result of basal cytotoxicity distributed to organs (1, 2);
4. as a more pragmatic aim of the programme, the selection of combinations of tests with the best prediction of human acute toxicity (not necessarily combinations of the best tests), to make up batteries for potential use as adjuncts or alternatives to standard animal tests. This requires soft (unbiased) multivariate modelling. Stage I modelling will naturally only result in prediction of human toxic blood concentrations (1, 2, 4); and
5. the incorporation of *in vitro* toxicity data into more-complicated Stage II kinetic models (1, 2, 4), to permit the selection of batteries of *in vitro* tests for predicting human toxic dosage.

This paper will focus on the first three aims. A core of the collected *in vitro* and *in vivo* data (5–9) will be used for a survey analysis, based only on group-specific data. To achieve the second aim, the results from the 61 assays used to test all 50 reference chemicals will be compared with three sets of human lethal concentrations (clinical lethal concentrations, forensic lethal concentrations, and peak concentrations from approximate LC50 curves) by univariate linear regression analysis. These comparisons will include preliminary toxicokinetic modelling and an analysis of outlier chemicals in the correlations for different groups of assays. To achieve the third aim, the most predictive *in vitro* results in the previous study will be compared with the best fitting set of the three human lethal concentrations. Here, an analysis of groups of chemicals with similar action will be compared, including an analysis of outlier chemicals. Correlations for the groups and individual outliers will be compared with known effects and mechanisms.

The goal of the present *in vitro/in vivo* evaluations is to give a robust overview of the relevance of cytotoxicity tests for human acute toxicity, which would permit testing of the first three basal cytotoxicity hypotheses and also provide a necessary platform for the further, more-refined studies. Hence, this paper is the most pivotal part of the evaluation, covering most aims of the MEIC programme.

The four other forthcoming papers in the series will use larger parts of the collected database (remaining test results, complete approximate LC50 curves, and more kinetic data) for supplementary evaluations. The first of these papers will focus on multivariate soft PLS modelling leading to battery selection and optimisation. The relative predictions by each of the 61 assays will be presented in that paper. In the second paper, a thorough analysis of the relationship between the human approximate LC50 curve over time and *in vitro* toxicity at different exposure times will be performed for each individual chemical. Relevant *in vitro* results will be pinpointed on a case-by-case basis, and thus the study will avoid errors due to statistical analysis of groups of chemicals. In this analysis, all the specific characteristics for each individual chemical with possible connections to, or influence on, the *in vitro/in vivo* correlation will be considered. Such characteristics are all lethal symptoms (not only group-specific patterns of symptoms), time-frames for the symptoms, accumulation of the chemical in organs, and blood protein binding. In this second paper, the authors will try to convert the human LC50 curves to corresponding dosages, in order to assess the practicability of *in vitro* test batteries for the prediction of lethal dosage. The third paper will report the findings of a study using the fine-tuned information from the second paper to select batteries of tests by standard multiple linear regression techniques, this time including an opti-

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mal blood–brain barrier-factor. The fourth paper will present an evaluation of the predictability of the remaining results from all the methods which only tested subsets of the 50 chemicals (6–8).

Methods and Results

The first general method compares analogous toxicity from different species (rat, mouse and human) or *in vitro/in vivo*, for the 50 reference chemicals with linear regression analysis, i.e. doses are compared to doses and concentrations to concentrations, and similar exposure times between compared toxicities are used.

In this study, *in vitro* IC50 values (geometric average 50% inhibitory concentrations from cytotoxicity tests [6]) have been compared with human mean lethal serum concentrations. The goal of the IC50/lethal concentration comparisons is to compare the concentrations available to the cells (and other *in vitro* test objects) in culture, i.e. the free fraction *in vitro*, with the concentration of the same chemical reaching cells in the human post-capillary extracellular fluid, i.e. the free fraction of the chemical in serum.

Since neither of the two free fractions are usually measured, the IC50 and lethal concentrations in this study have to represent these fractions in the comparisons. For the majority of chemicals in the study with low or moderate (0–75%) blood protein binding at lethal dosage (see Table V of Part V [9]), this is adequate, due to the similar serum contents (5–10%) of a typical human 24-hour cell line test (see Table II of Part IV [8]) and the human serum content of plasma plus extracellular fluid (20%). However, for strong (> 90%) blood protein bound chemicals, the IC50 is a better model of the free fractions when compared with the human serum concentrations and will underestimate the lethal concentration (see Discussion).

Lethal concentrations, or rather, the free fractions they represent, ought to be measured after the distribution of chemicals into cells, if they are to be equivalent to IC50 values for chemicals with a high accumulation in cells. Measurement of the lethal concentration at, or after, peak concentrations for all chemicals in the study, will ensure the comparability of most data. However, for a few chemicals with a high distribution volume (see Table V of Part V [9]) and an unrecognised incomplete distribution to target cells at peak concentrations, the comparability of data may be less good (see Discussion).

Linear regression was once chosen for these comparisons (1), because it is especially suitable for measuring the relationship between two continuous and linear variables (1, 12). In addition, the results of linear regression, in the form of R^2, are familiar to most researchers and are easy to interpret ($R^2 \times 100$ roughly corresponds to percentage prediction). However, there are some drawbacks with linear regression. The fit between two sets of data are determined by R^2, as well as the slope and the intercept. Hence, R^2 alone cannot be used to compare the predictions of different sets of data, and the whole regression formula must be used. Each comparison has two regression formulae, depending on how the independent (x) and the dependent (y) variables are defined. To permit comparisons of regression formulae, the potentially predictive data, for example, IC50 values, have always been denoted by x, and data to be predicted, for example, human lethal concentrations, denoted by y.

The second general method defines the outlier chemicals in the various comparisons, as a first step toward explaining outlier behaviour in mechanistic or toxicokinetic terms. To facilitate comparisons of outliers between data sets, deviations between predictive data and predicted data have been based on a direct comparison of raw data in the tables (relating them to a slope of 1 and an intercept of 0), rather than on the regression line of each individual plot. Thus, an outlier chemical (in this absolute and comparable sense) may be less outlying than some other chemicals in the context of each separate plot.

The third general method compares the correlations found for groups of chemicals, as well as deviating predictions for outlier chemicals, with effects, kinetics and mechanisms of action of the chemicals (the mechanistic part of the correlative/mechanistic approach). The results of this method are a coupling of correlative results with qualitative toxicological knowledge, in order to understand the underlying causes for the gross group correlations and the deviating predictions.

When all these methods are used to evaluate data, it is important to keep the margins of error of the raw data in mind, because they determine the discriminatory power of the comparisons. The LD50 values from different experiments are known to vary, and it is not unreasonable to assume that the lowest known value (the LD50 value used in this study) for some chemicals may be one log step lower than other, potentially more correct, published values. Likewise, most of the human lethal doses and the standard lethal concentrations (clinical and forensic) of this study are average values from arithmetically similar values, which does not exclude a potential error of at most one log step for some of them (9). The peak values from the approximate LC50 curves are sometimes averages of LC0 and LC100 curves differing by several log steps. Peak values from individual LC0 or LC100 curves for some chemicals with only a few case reports are even more uncertain. For reasons already given, the human lethal peak concentrations of ten chemicals, i.e. diazepam, digoxin, malathion, xylene, nicotine, warfarin, barium nitrate, verapamil hydrochloride, sodium oxalate and atropine sulphate (chemicals 4, 6, 15, 17, 18, 31, 37, 40, 46 and 49) were noted as being especially uncertain in Part V (9). IC50 values from *in vitro* tests in the same system often have a surprising arithmetic precision, but pooled values from several assays, as used in this study, are derived from results varying by one, or even two, log steps for some chemicals (5–8).

Inaccurate comparisons of IC50 and lethal concentration for some chemicals due to toxicokinetic factors must be added to these margins of error of raw data. As discussed above, two such main errors may be: a) an inaccurate lethal concentration for chemicals with a high accumulation in cells (high distribution volumes) due to measurement of the concentration before complete distribution to human target cells; or b) an excessively low IC50 for a chemical with very strong protein binding at lethal dosage (see above and Discussion). The overprediction of the human lethal concentration value by the IC50 values for the latter group of chemicals will be most accentuated in protein-free systems (aquatic ecotoxicological systems), but probably will also affect 5–10% serum protein, human cell systems. As judged from the incomplete human blood protein binding data in Table V of Part V (9), the second group of chemicals may include iron (II) sulphate, diazepam, malathion, 2,4-dichlorophenoxyacetic acid, nicotine, copper (II) sulphate, mercury (II) chloride, thioridazine hydrochloride, warfarin, hexachlorophene, pentachlorophenol and verapamil hydrochloride (chemicals 3, 4, 15, 16, 18, 27–29, 31 and 38–40). Note that only hexachlorophene has a documented high protein binding at overdosage (asterisked in Table V of Part V [9]), while the other chemicals only have low-dose binding data, which are probably not valid for the high lethal concentrations.

To summarise the effects of uncertainties of the raw data and uncertainties of data due to toxicokinetic incongruencies, a variation for one specific chemical of less than one log step between compared *in vitro* and *in vivo* values is unlikely to always be significant, while a larger difference ought to have a definitive discriminative value.

The main purpose of the previous parts of this series (5–9) was to provide the present study with base data. In general, raw toxicity data used in the comparisons are not reiterated in the present paper, and can be found in the tables of the previous articles (notably references 6–9). The method numbers in the present article refer to those of Tables I–VII of Part IV (8). Table I of Part IV (8) can be used to convert the numbers of methods to the original Part II and Part III numbers. The table also gives details of sourcing data not published in Part IV.

Prediction by rat and mouse LD50 values

To form a base-line for judging the relative efficiencies of *in vitro* tests and standard animal tests in the prediction of human acute toxicity, the human, rat and mouse acute oral lethal doses listed in Table VII of Part V (9) were compared.

As shown in Figure 1, the prediction of mouse LD50 by rat LD50 is good ($R^2 = 0.85$, slope = 0.88). Mouse toxicity was clearly underpredicted by rat LD50 for three chemicals (nicotine, chloroform and diphenylhydantoin [chemicals 18, 33 and 44]). Clear underprediction is defined as a difference of one log step or more between LD50 values, as calculated directly from Table VII of Part V (9).

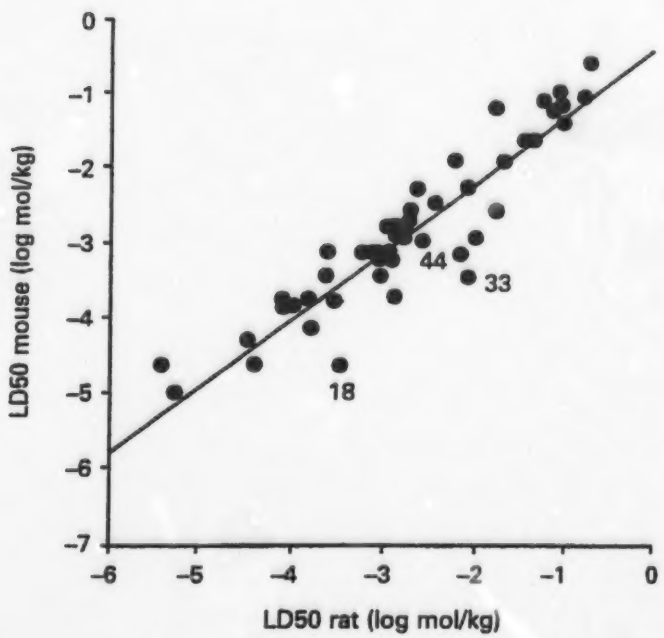
As shown in Figure 2, the prediction of human acute lethal dose by rat LD50 is not

good (R^2 = 0.61, slope = 0.83). As demonstrated in Figure 2 and Table I, there are seven clear outlier chemicals, i.e. chemicals with differences between rat LD50 and human lethal dose of one log step or more, as judged from direct comparisons of the data in Table VII of Part V (9). These chemicals are digoxin, nicotine, mercury (II) chloride, thioridazine hydrochloride, warfarin, sodium oxalate and atropine sulphate (chemicals 6, 18, 28, 29, 31, 46 and 49).

As shown in Figure 3, the prediction of human lethal dose by mouse LD50 is better (R^2 = 0.65, slope = 0.90) than the rat LD50 prediction. According to the stated criteria, six outlier chemicals were found, as indicated in Figure 3 and Table I. These chemicals were digoxin, dextropropoxyphene hydrochloride, warfarin, chloroform, sodium oxalate and atropine sulphate (chemicals 6, 22, 31, 33, 46 and 49).

The crude analysis of outlier chemicals in Table I reveals that both rodent species overtly underpredict the human lethal dose for digoxin, sodium oxalate and atropine sulphate, and also overpredict the human lethal dose for warfarin. This is in line with the good correspondence (R^2 = 0.85) between the LD50 values from the two rodent species. Another common trend of false predictions of human lethal dose by rodent LD50 values is shown in Figures 2 and 3, that the effects of more-toxic compounds seem to be less well predicted than those of less-toxic compounds (chloroform and sodium oxalate being exceptions).

Figure 1: Rat oral LD50 values (log mol/kg) for the 50 MEIC reference chemicals plotted against mouse oral LD50 values for the same chemicals



Original data are from Table VII of Part V (9). Corresponding raw data in mg/kg are found in Table I of Part V (9).

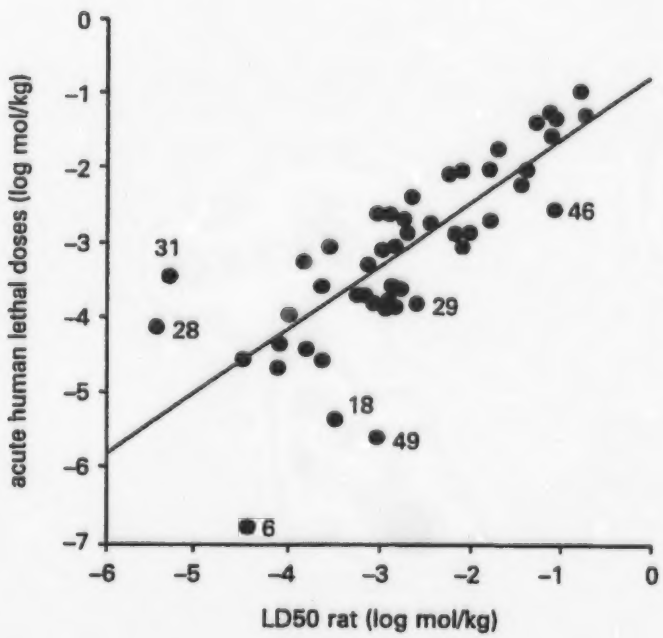
$y = 0.880x - 0.471$; $R^2 = 0.852$.

Prediction by 61 in vitro toxicity tests

Table II shows R^2 values and slopes from linear regression comparisons between IC50 values in logM, from 61 *in vitro* toxicity assays and three independent sets of human acute lethal blood concentrations. The *in vitro* results are to be found in Tables I and III–V of Part II (6), Table II of Part III (7), and Tables III–VII and IX of Part IV (8). The human lethal concentrations, i.e. clinical lethal concentrations, forensic lethal concentrations and peak lethal concentrations, are found in Table VIII of Part V (9). Table II also shows the number of chemicals (n) tested by each test, which is a prerequisite to judging the outcome (R^2 value) of the comparisons, i.e. too many missing chemicals would invalidate the R^2 value.

It can be seen in Table II that *in vitro* predictions of clinical and forensic lethal concentrations are relatively similar for each test. The prediction of peak lethal concentrations by each test is often better than the prediction of clinical and forensic lethal concentrations. It is also seen that tests belonging to the same group often give similar degrees of prediction of human lethal concentrations. As judged from the mean values and ranges of R^2, human cell line assays seem to give the best predictions of peak lethal concentrations (mean R^2 = 0.64,

Figure 2: Rat oral LD50 values (log mol/kg) for the 50 MEIC reference chemicals plotted against human average oral acute lethal doses for the same chemicals



Original rat LD50 values and human lethal dose values are from Table VII of Part V (9). Corresponding rat LD50 values in mg/kg are found in Table I of Part V (9) and human LD50 values in mg/kg are found in Table II of Part V (9).

$y = 0.829x - 0.877$; $R^2 = 0.607$.

Table I: Chemicals with clearly false predictions of human lethal dose by rodent LD50

Number	Chemical	Rat oral LD50	Mouse oral LD50
6.	Digoxin	−−	−−
18.	Nicotine	−	
22.	Propoxyphene hydrochloride		−
28.	Mercury (II) chloride	+	
29.	Thioridazine hydrochloride	−	
31.	Warfarin	+	+
33.	Chloroform		+
46.	Sodium oxalate	−	−
49.	Atropine sulphate	−−	−−

False negative predictions: − = rodent LD50 1 log higher than the human oral lethal dose; −− = rodent LD50 2 log higher than the human oral lethal dose.

False positive predictions: + = rodent LD50 1 log lower than the human oral lethal dose.

range 0.52–0.80). Animal cell line assays and primary cultures seem to be somewhat less predictive (mean R^2 = 0.52 and 0.58, range 0.41–0.65 and 0.46–0.70, respectively). Note that the high R^2 values for some animal primary cultures (methods 38–40) are less reliable, since they have been obtained from tests on relatively few chemicals. The fish cell line assays are as predictive as the non-human mammalian cell line assays (mean R^2 = 0.52, range 0.24–0.60). The non-fish ecotoxicological tests generally give a modest prediction (mean R^2 = 0.36, range 0.27–0.51). The few tests with human primary cultures and ovalbumin are difficult to compare with the other tests, because of many missing *in vitro* data. However, the mean R^2 of 0.46 for the ovalbumin tests approaches the prediction given by cellular systems.

The data in Table III were calculated to enable a comparison of collective prediction by groups and subgroups of tests, and also to provide a base for a coherent and robust analysis of outlier chemicals (it is difficult to provide such an analysis based on comparisons of individual tests). Two groups of tests in Table II were not included in Table III, because of few methods in each group and the many missing chemicals, i.e. the human primary cultures and the cell-free systems (methods 19, 20, 60 and 61). Instead, eight subgroups to the main groups of Table II have been included, with further discrimination of tests in terms of different exposure times or test subjects.

In Table III, the first row of data for each group are slopes and R^2 values from linear regression comparisons between average IC50 values for each chemical from all the assays in the group and the three sets of human lethal concentrations from Table VIII of Part V (9). The second row of data for each group are slopes and R^2 values from comparisons between average IC50 values, divided by a factor of ten for all 32 chemicals freely entering the brain (see Table V of Part V), and human lethal concentrations.

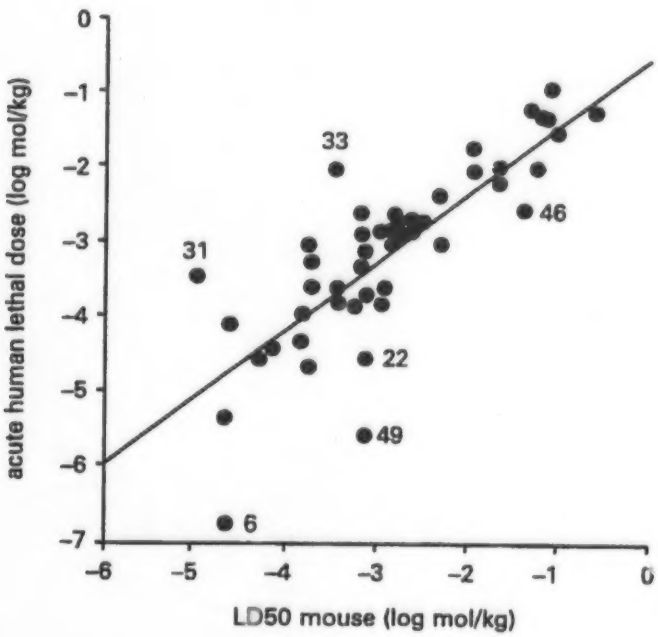
From a comparison between mean R^2 values for the five largest groups in Table II (human cell lines, animal cell lines, animal primary cultures, fish cell cultures, and other ecotoxicological systems) and R^2 values for the collective prediction of clinical lethal concentrations, forensic lethal concentrations and peak concentrations for the same groups in Table III, it can be seen that collective prediction is generally better than the prediction of an average individual test. The R^2 values of Table III are often about ten times larger than mean R^2 values for corresponding groups and predictions in Table II. The superior prediction by average IC50 values from all the tests in a group, when compared to predictions of individual

tests, is further accentuated if the numbers of chemicals included in the prediction are considered. All but one of the collective predictions in Table III are made for all 50 reference chemicals, while most predictions by individual tests in Table II include fewer compounds.

Apart from this, the collective prediction of clinical lethal concentrations, forensic lethal concentrations and peak concentrations by various groups of tests in Table III follow the same pattern as the prediction by individual tests in Table II. As judged by the R^2 values, most groups predict peak concentrations better than clinical and forensic lethal concentrations. Clinical and forensic lethal concentrations are predicted approximately equally by most groups of tests. Phylogenetically higher systems predict human lethal concentrations better than lower systems. Thus, the R^2 values for the prediction of peak concentrations are 0.74 for human cell lines, 0.64 for animal cell lines, 0.65 for animal primary cultures, 0.57 for fish cell cultures, and 0.46 for non-fish ecotoxicological systems.

The average *in vitro* IC50 values were divided by ten for 32 chemicals which appear to cross the blood–brain barrier freely (9). This represents "hard modelling", and was made to test a hypothesis about the existence of a ten-fold increased vulnerability of brain vital functions in comparison with general cytotoxicity, as found in previous studies (10, 11, 13). The factor of ten was chosen because of this hypothesis.

Figure 3: Mouse oral LD50 values (log mol/kg) for the 50 MEIC reference chemicals plotted against human average oral acute lethal doses for the same chemicals



Original mouse LD50 values and human lethal dose values are from Table VII of Part V (9). Corresponding mouse LD50 values in mg/kg are found in Table I of Part V (9) and human LD50 values in mg/kg are found in Table II of Part V (9).

$y = 0.902x - 0.551$; $R^2 = 0.653$.

Table II: IC50 values from 61 *in vitro* assays compared with human lethal concentrations (LCs)

No.	Method	n	Clinical LC		Forensic LC		Peaks from LC50 curves	
			Slope	R^2	Slope	R^2	Slope	R^2
Human cell lines								
1.	Hep G2, protein content, 24 hours	45	0.92	0.62	0.87	0.63	0.90	0.68
2.	Hep G2, protein content, 24 hours	45	0.74	0.61	0.73	0.64	0.73	0.66
3.	Hep G2, MTT, 24 hours	48	0.91	0.56	0.87	0.57	0.91	0.63
4.	Hep G2, morphology, 24 hours	45	0.69	0.64	0.65	0.61	0.69	0.71
5.	Chang, morphology, 24 hours	45	0.73	0.68	0.69	0.67	0.71	0.73
6.	HeLa, morphology, 24 hours	49	0.61	0.52	0.61	0.59	0.59	0.54
7.	Hep 2, protein content, 24 hours	48	0.89	0.57	0.86	0.58	0.90	0.64
8.	Hep 2, LDH release, 24 hours	44	0.89	0.61	0.85	0.61	0.90	0.67
9.	HL-60, ATP content, 24 hours	45	0.87	0.66	0.84	0.66	0.87	0.72
10.	HFL1, MTT, 24 hours	49	0.81	0.48	0.80	0.51	0.80	0.52
11.	SQ-5, LDH content, 48 hours	47	0.74	0.57	0.72	0.59	0.71	0.59
12.	SQ-5, killing index, 48 hours	44	0.77	0.59	0.74	0.62	0.76	0.63
13.	NB-1, protein content, 48 hours	37	0.75	0.58	0.71	0.58	0.74	0.65
14.	McCoy, morphology/trypan blue exclusion, 72 hours	48	0.73	0.50	0.68	0.48	0.70	0.54
15.	Hep G2, morphology/pH changes, 168 hours	45	0.60	0.58	0.57	0.58	0.60	0.65
16.	Chang, morphology/pH changes, 168 hours	45	0.65	0.63	0.63	0.64	0.63	0.68
17.	HeLa, pH changes, 168 hours	49	0.54	0.48	0.53	0.52	0.52	0.50
18.	MRC-5, protein content, 6 weeks	45	0.80	0.75	0.76	0.77	0.78	0.80
Mean:			0.76	0.59	0.73	0.60	0.75	0.64
Human primary cultures								
19.	PMN leukocytes, viable cell count, 3 hours	36	0.69	0.45	0.68	0.49	0.70	0.50
20.	PMN leukocytes, chemotaxis, 3 hours	39	0.69	0.45	0.68	049	0.69	0.50
Animal cell lines								
21.	ELD, ATP leakage, 10 minutes	37	0.56	0.36	0.51	0.34	0.58	0.41
22.	ELD, ATP leakage, 10 minutes	34	0.65	0.40	0.62	0.43	0.67	0.47
23.	HTC, macromolecular content, 24 hours	48	0.52	0.33	0.49	0.34	0.56	0.41
24.	L2, [^{3}H]-proline uptake, 24 hours	50	0.71	0.37	0.68	0.37	0.71	0.40
25.	3T3, MTT, 24 hours	48	0.77	0.48	0.70	0.44	0.77	0.54
26.	LCC-PK1, protein content, 24 hours	44	0.70	0.60	0.68	0.62	0.67	0.62
27.	BP8, cell number, 48 hours	50	0.74	0.48	0.73	0.51	0.73	0.50
28.	PC12h, protein content, 48 hours	40	0.66	0.50	0.63	0.54	0.64	0.54
29.	MDBK, morphology/trypan blue exclusion, 72 hours	48	0.77	0.56	0.73	0.55	0.72	0.57
30.	Hepa-1, protein content, 72 hours	50	0.65	0.48	0.64	0.53	0.63	0.50

Table II: continued

No.	Method	n	Clinical LC Slope	Clinical LC R^2	Forensic LC Slope	Forensic LC R^2	Peaks from LC50 curves Slope	Peaks from LC50 curves R^2
31.	3T3-L1, protein content, 72 hours	48	0.76	0.61	0.70	0.56	0.75	0.65
32.	Balb 3T3, colony formation, 168 hours	43	0.79	0.59	0.78	0.61	0.79	0.64
Mean:			0.69	0.48	0.66	0.49	0.69	0.52
Animal primary cultures								
33.	Rat skeletal muscle cells, contractility, 1 hour	49	0.46	0.38	0.42	0.35	0.48	0.46
34.	Chick neurons, NRU, 20 hours	50	0.71	0.52	0.68	0.52	0.67	0.51
35.	Chick neurons, MTT, 24 hours	49	0.83	0.55	0.78	0.54	0.78	0.55
36.	Rat hepatocytes, MTT, 24 hours	48	0.85	0.53	0.77	0.50	0.84	0.58
37.	Rat hepatocytes, morphology/ trypan blue/LDH, 24 hours	48	0.71	0.50	0.68	0.50	0.68	0.53
38.	Rabbit erythrocytes, ATP content, 24 hours	38	0.76	0.61	0.69	0.57	0.74	0.60
39.	Rat skeletal muscle cells, CK depletion, 24 hours	38	0.75	0.57	0.70	0.58	0.80	0.67
40.	Rat skeletal muscle cells, glucose consumption, 24 hours	32	0.90	0.64	0.88	0.68	0.88	0.70
41.	Rat skeletal muscle cells, contractility, 24 hours	49	0.66	0.55	0.62	0.54	0.67	0.64
Mean:			0.74	0.54	0.69	0.53	0.73	0.58
Fish cell cultures								
42.	Fish hepatocytes, ^{86}Rb-leakage, 3 hours	42	0.42	0.27	0.44	0.35	0.37	0.24
43.	R1, NRU, 24 hours	45	0.74	0.49	0.72	0.50	0.74	0.53
44.	R1, protein content, 24 hours	45	0.75	0.51	0.72	0.52	0.75	0.57
45.	R1, protein content, 144 hours	45	0.73	0.54	0.71	0.56	0.73	0.60
46.	RTG-2, protein content, 48 hours	45	0.79	0.54	0.77	0.56	0.79	0.59
47.	RTG-2, NRU, 48 hours	46	0.78	0.51	0.76	0.53	0.78	0.56
48.	RTG-2, ATP content, 48 hours	45	0.74	0.52	0.70	0.54	0.75	0.57
Mean:			0.71	0.48	0.69	0.51	0.70	0.52
Other ecotoxicological systems								
49.	*Photobacterium phosphoreum*, bioluminescence, 5 minutes	44	0.42	0.28	0.41	0.30	0.40	0.27
50.	*Photobacterium phosphoreum*, bioluminescence, 5 minutes	45	0.55	0.37	0.54	0.38	0.55	0.40

Table II: continued

No.	Method	n	Clinical LC		Forensic LC		Peaks from LC50 curves	
			Slope	R^2	Slope	R^2	Slope	R^2
51.	*Escherichia coli*, growth, 18 hours	42	0.47	0.32	0.50	0.41	0.47	0.36
52.	*Halobacterium halobium*, growth, 120 hours	41	0.41	0.27	0.42	0.32	0.43	0.32
53.	*Daphnia magna*, immobilisation, 24 hours	44	0.60	0.50	0.54	0.45	0.58	0.51
54.	*Daphnia magna*, immobilisation, 24 hours	49	0.54	0.42	0.47	0.34	0.53	0.44
55.	*Brachionus calyciflorus*, mortality, 24 hours	47	0.33	0.33	0.32	0.37	0.30	0.31
56.	*Artemia salina*, mortality, 24 hours	47	0.42	0.30	0.43	0.36	0.41	0.31
57.	*Streptocephalus proscideus*, mortality, 24 hours	49	0.46	0.41	0.46	0.48	0.44	0.40
58.	Pollen tubes, growth 18 hours	49	0.51	0.26	0.47	0.27	0.52	0.29
59.	*Allium cepa*, growth, 72 hours	49	0.47	0.33	0.48	0.38	0.45	0.33
Mean:			0.47	0.34	0.46	0.37	0.46	0.36
Cell free systems								
60.	Ovalbumin, protein denaturation, 2 hours	33	0.63	0.36	0.60	0.38	0.68	0.45
61.	Ovalbumin, protein denaturation, 2 hours	33	0.64	0.38	0.62	0.40	0.68	0.47

CK = creatine kinase; LDH = lactate dehydrogenase; NRU = neutral red uptake; PMN = polymorphonuclear.

As seen in Table III, the use of this factor (blood–brain barrier compensation) improves the prediction of human lethal concentrations. The improvement is generally small for the prediction of clinical and forensic lethal concentrations, but is considerable for the prediction of peak concentrations. Thus, R^2 for the main groups of tests (human cell line assays, animal cell line assays, animal primary cultures, fish cell assays, and non-fish ecotoxicological tests, respectively) increases to 0.84, 0.70, 0.69, 0.67, and 0.52, when the prediction of peak concentrations includes this simple algorithm. The use of blood–brain barrier compensation also increases the slopes of the regression formulae for the five main groups of tests from 0.86, 0.85, 0.83, 0.78 and 0.62, to 0.97, 0.93, 0.88, 0.91 and 0.70, respectively.

Outlier chemicals in the comparisons between blood–brain barrier-compensated average IC50 values from ten groups of tests and human peak concentrations are presented in Table IV. The comparison of blood–brain barrier-compensated IC50 values and peaks was chosen for this analysis, as it represented the best prediction of human lethal concentrations by the groups (except for bacteria) and thus potentially would lead to a minimal number of outliers. Outlier chemicals were identified by a direct comparison of raw data, to facilitate comparisons between groups as well as between *in vitro* and rodent LD50 predictions. A broad criterion for false prediction (one log step) was deliberately chosen to achieve robustness and simplification, at this stage of the evaluation.

As shown in Table IV, the number of outliers increases in proportion to decreasing

Table III: Average IC50 value from 13 groups of *in vitro* assays compared with human lethal concentrations (LCs)

Group of *in vitro* assays (average of method numbers in brackets)	n	Clinical LC[a]		Forensic LC[b]		Peaks from LC50 curves[c]	
		Slope	R^2	Slope	R^2	Slope	R^2
Human cell lines, all (1–18)	50	0.87 0.95	0.69 0.73[d]	0.84 0.91	0.71 0.75[d]	0.86 0.97	0.74 0.84[d]
Human cell lines, 24 hours (1–10)	50	0.91 0.97	0.69 0.71[d]	0.89 0.94	0.71 0.73[d]	0.91 1.00	0.74 0.82[d]
Human cell lines, 48 hours (11–13)	48	0.78 0.74	0.62 0.51[d]	0.75 0.71	0.63 0.52[d]	0.76 0.75	0.64 0.58[d]
Human cell lines, 72 hours–6 weeks (14–18)	50	0.76 0.82	0.68 0.71[d]	0.73 0.79	0.69 0.72[d]	0.75 0.84	0.72 0.81[d]
Animal cell lines, all (21–32)	50	0.86 0.90	0.59 0.60[d]	0.82 0.85	0.59 0.60[d]	0.85 0.93	0.64 0.70[d]
Animal cell lines, 24 hours (23–26)	50	0.79 0.85	0.52 0.54[d]	0.75 0.80	0.51 0.53[d]	0.79 0.89	0.57 0.65[d]
Animal primary cultures (33–41)	50	0.84 0.86	0.60 0.60[d]	0.79 0.80	0.59 0.57[d]	0.83 0.88	0.65 0.69[d]
Animal primary cultures with basal cytotoxicity endpoints (34–40)	50	0.88 0.95	0.60 0.62[d]	0.82 0.89	0.58 0.60[d]	0.85 0.96	0.62 0.70[d]
Fish cell cultures, all (42–48)	50	0.79 0.88	0.53 0.57[d]	0.77 0.86	0.55 0.59[d]	0.78 0.91	0.57 0.67[d]
Non-fish ecotoxicological systems (49–59)	50	0.64 0.69	0.45 0.46[d]	0.62 0.66	0.46 0.47[d]	0.62 0.70	0.46 0.52[d]
Small multicellular aquatic organisms (53–57)	50	0.55 0.59	0.43 0.45[d]	0.52 0.56	0.43 0.44[d]	0.53 0.59	0.43 0.48[d]
Plants (58, 59)	50	0.57 0.66	0.34 0.38[d]	0.57 0.65	0.37 0.41[d]	0.56 0.68	0.36 0.44[d]
Bacteria (49–52)	50	0.59 0.51	0.35 0.27[d]	0.58 0.51	0.37 0.30[d]	0.60 0.56	0.39 0.36[d]

[a]*Mean values compiled from ten clinical handbooks (9).*

[b]*Mean values compiled from eight forensic medicine lists (9).*

[c]*From published LC50 curves over time drawn from plots of time-related high survival and lethal blood concentrations from human acute poisoning cases, obtained in the MEMO study (9).*

[d]*IC50 was divided by ten for all 32 MEIC reference chemicals known to pass the blood–brain barrier freely, to compensate for a hypothetical extrasensitivity of the brain to cytotoxicity (i.e. extraordinary short-term loss of vital functions of the brain) compared to most other organs (liver, kidney, etc.).*

Table IV: Chemicals with clearly false predictions of human peak concentrations by blood–brain barrier-compensated average IC50 values from ten groups of *in vitro* assays

No. Chemical	Human cell lines (24 hours) Methods 1–10	Human cell lines, all (24 hours–6 weeks) Methods 1–18	Animal cell lines (24 hours) Methods 23–26	Animal cell lines, all (10 minutes–168 hours) Methods 21–32	Animal primary cultures with basal cytotoxicity endpoints Methods 34–40	Fish cell cultures (3–144 hours) Methods 42–48	Non-fish ecotoxicological systems, all (5 minutes–72 hours) Methods 49–59	Small multicellular aquatic organisms (24 hours) Methods 53–57	Plants[a] (18 hours and 72 hours) Methods 58 and 59	Bacteria (5 minutes–120 hours) Methods 49–52
1. Paracetamol								+		
2. Acetylsalicylic acid									+	
3. Iron (II) sulphate										–
4. Diazepam									+	
5. Amitriptyline hydrochloride										
6. Digoxin	–		--	--	---	---	---	--	--	----
7. Ethylene glycol						–				–
8. Methanol										
9. Ethanol			+							
10. Isopropanol			+							
11. 1,1,1-Trichloroethane										
12. Phenol								+		
13. Sodium chloride										
14. Sodium fluoride							–	–		--
15. Malathion	–	–			–	–			–	–
16. 2,4-Dichlorophenoxyacetic acid							+		+++	
17. Xylene									+	
18. Nicotine			–			–				
19. Potassium cyanide							+	+	++	
20. Lithium sulphate										–
21. Theophylline									+	
22. Dextropropoxyphene hydrochloride										

23. Propranolol hydrochloride									
24. Phenobarbital									
25. Paraquat					–				
26. Arsenic trioxide									– –
27. Copper (II) sulphate						+	++		
28. Mercury (II) chloride		+	+	+	+	+	+	++	+	++
29. Thioridazine hydrochloride		+	+				+	+		
30. Thallium sulphate	–		–			–			+	–
31. Warfarin									+	
32. Lindane							–			– –
33. Chloroform									+	
34. Carbon tetrachloride			–	–		–	–	–		–
35. Isoniazid									+	
36. Dichloromethane										–
37. Barium nitrate										
38. Hexachlorophene		+	+	+	+	+	+	++++	++	
39. Pentachlorophenol							+	++	++	+
40. Verapamil hydrochloride										–
41. Chloroquine phosphate										
42. Orphenadrine hydrochloride								+		
43. Quinidine sulphate										
44. Diphenylhydantoin				+				+		
45. Chloramphenicol										+
46. Sodium oxalate										– –
47. Amphetamine sulphate										– –
48. Caffeine										
49. Atropine sulphate			–	–		–	–		–	– –
50. Potassium chloride										

[a]*Chloroplast-free organs of plants.*

False negative prediction of blood–brain barrier-compensated IC50 value 1 log higher (–), 2+ log higher (– –), 3+ log higher (– – –), or 4+ log higher (– – – –) than the human lethal peak concentration.

False positive prediction of blood–brain barrier-compensated IC50 value 1 log lower (+), 2+ log lower (++), 3+ log lower (+++), or 4+ log lower (++++) than the human lethal peak concentration.

predictions (lower R^2 and slopes) of the peak concentrations by the various groups of tests, as would be expected. For the five main groups discussed previously (human cell line assays, animal cell line assays, animal primary cultures with basal cytotoxicity endpoints, fish cell cultures and non-fish ecotoxicological tests), the numbers of overt outliers are four, six, four, ten and twelve, respectively. A few outlier chemicals were badly predicted by most *in vitro* systems, including human cell cultures, i.e. digoxin, malathion, mercury [II] chloride, thioridazine hydrochloride, thallium sulphate and hexachlorophene (chemicals 6, 15, 28–30 and 38). Of these outliers, three represent underpredictions *in vitro* (digoxin, malathion and thallium sulphate), and three represent overpredictions (mercury [II] chloride, thioridazine sulphate and hexachlorophene). A few other outliers were badly predicted by animal and fish cells, but not by human cells, i.e. nicotine, carbon tetrachloride and atropine sulphate (chemicals 18, 34 and 49).

The non-fish ecotoxicological tests resulted in a marked increase in the numbers of outlier chemicals, when compared with the vertebrate cell tests. The three subgroups, i.e. aquatic organisms, non-photosynthesising organs of plants (simply called plants) and bacteria, also resulted in different patterns of outliers. When the nine previously discussed outliers were excluded, and when the degree of outlier behaviour (differences in potencies) was accounted for, these patterns were as follows. The aquatic organisms overpredicted the blood–brain barrier-compensated human peak concentrations of paracetamol, phenol, potassium cyanide, copper (II) sulphate, mercury (II) chloride, hexachlorophene, pentachlorophenol, orphenadrine hydrochloride and diphenylhydantoin (chemicals 1, 12, 19, 27, 28, 38, 39, 42 and 44), and underpredicted the human toxicity of sodium fluoride (chemical 14). The plant systems overpredicted the human toxicities of acetylsalicylic acid, diazepam, 2,4-dichlorophenoxyacetic acid, xylene, potassium cyanide, theophylline, thallium sulphate, warfarin, chloroform, isoniazid, hexachlorophene and pentachlorophenol (chemicals 2, 4, 16, 17, 19, 21, 30, 31, 33, 35, 38 and 39). The bacterial systems overpredicted the human toxicities of mercury (II) chloride, pentachlorophenol and chloramphenicol (chemicals 28, 39 and 45) and underpredicted the human toxicities of iron (II) sulphate, digoxin, ethylene glycol, sodium fluoride, lithium sulphate, arsenic trioxide, lindane, barium nitrate, verapamil hydrochloride, sodium oxalate and amphetamine sulphate (chemicals 3, 6, 7, 14, 20, 26, 32, 37, 40, 46 and 47).

Prediction of human lethal concentrations by 24-hour human cell line assays

To assess the relevance of the best MEIC assays, as described in the third aim in the Introduction, the group of 24-hour human cell line assays was selected for further evaluation. The 24-hour cell line tests were chosen because they shared the best direct (not blood–brain barrier-compensated) prediction with the group of all human cell line assays, but were a better defined group. The 24-hour exposure time of the tests means that it is possible to make analogous comparisons of these tests to human concentrations with the same time-frames for effects and/or exposure, which cannot be made with the larger group of tests.

Figures 4–6 are linear regression plots of the comparisons between average IC50 values from the ten 24-hour human cell line assays and the three sets of human lethal concentrations, i.e. clinical concentrations (Figure 4), forensic concentrations (Figure 5) and peak concentrations from approximate LC50 curves (Figure 6a). These results are also shown in Table III. The average IC50 values used for chemicals 1–30 are shown in Table IV of Part III (7), and the IC50 values for chemicals 31–50 from methods 1–10 are found in Table III of Part IV (8). Clinical lethal concentrations were predicted well (R^2 = 0.69), forensic lethal concentrations were predicted somewhat better (R^2 = 0.71), and peak concentrations were still better predicted (R^2 = 0.74). Similarly to the plots of rodent LD50 prediction of human lethal dose (Figures 1–3), the prediction for less-toxic chemicals was better than for more-toxic chemicals.

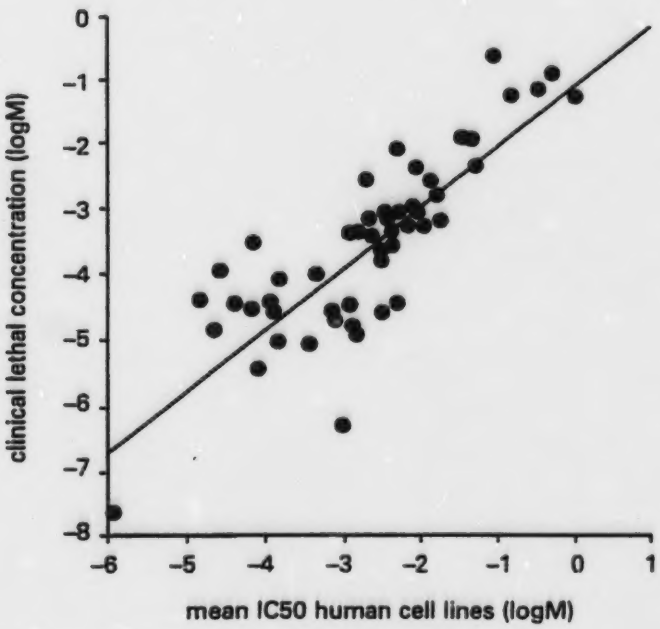
Figure 6b illustrates the short-term passage through the blood–brain barrier of overdose concentrations (9) of the chemicals shown in Figure 6a. A 1:1 line has been inserted instead of the regression line. It can be seen that chemicals with a restricted passage across the blood–brain barrier centre around the 1:1 line, while chemicals with free passage across the blood-brain barrier

centre around an imaginary line (around one log step) to the right of the 1:1 line. The prediction for all the chemicals would be improved if *in vitro* IC50 values for the 32 chemicals entering the brain freely were decreased about one logarithmic step, thereby centring all values around the 1:1 line. This simple algorithmic procedure can be called blood–brain barrier compensation (see above).

Figure 7 shows the results from such a blood–brain barrier compensation of the data in Figure 6. The prediction of human peak concentrations by blood–brain barrier-compensated average IC50 values from the 24-hour human cell line assays is clearly improved (R^2 = 0.82, slope 1.0). This good prediction is composed of predictions for each of the two groups of *in vitro* results, i.e. the direct prediction of peak concentrations for 18 chemicals with a restricted passage across the blood–brain barrier, and the indirect prediction with use of blood–brain barrier-compensation for 32 chemicals with free passage into brain.

To provide this analysis with a purely correlative robust base-line, no comparisons between correlations and qualitative toxicity

Figure 4: Mean IC50 values (logM) for the 50 MEIC reference chemicals from ten 24-hour human cell line assays (methods 1–10) plotted against clinically derived human lethal blood concentrations for the same chemicals



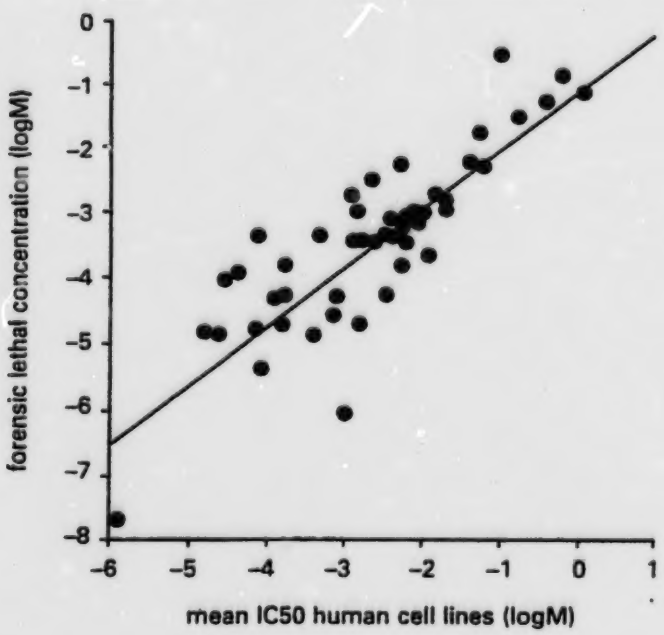
The clinical lethal concentration values derive from Table VIII of Part V (9). The average IC50 values from tests of chemicals 1–30 by methods 1–10 derive from Table VI of Part III (7). The average IC50 values from chemicals 31–50 tested by methods 1–10 have not been tabulated separately in the preceding papers, but individual IC50 values from tests of chemicals 31–50 by methods 1–10 can be found in Table III of Part IV (8).

$y = 0.914x - 1.201$; $R^2 = 0.686$.

data have been made so far. In order to explain the correlative findings in terms of chemical action, a series of comparisons between the correlative results and known effects and toxic mechanisms of the chemicals were performed.

To begin with, Table IX of Part V (9) was examined to try to detect a common pattern of lethal action of the 18 chemicals considered to have restricted access to the CNS. One common denominator was found, namely the absence of an early non-specific CNS excitation/depression, at the time of the peak concentrations of the chemicals. Fifteen of the 18 chemicals adhere to this pattern, i.e. acetylsalicylic acid, iron (II) sulphate, digoxin, 2,4-dichlorophenoxyacetic acid, lithium sulphate, arsenic trioxide, copper (II) sulphate, mercury (II) chloride, thallium sulphate, warfarin, barium nitrate, hexachlorophene, pentachlorophenol, verapamil hydrochloride and quinidine sulphate (chemicals 2, 3, 6, 16, 20, 26–28, 30, 31, 37–40 and 43). However, three chemicals, i.e. sodium chloride, sodium fluoride and sodium oxalate (chemicals 13, 14 and 46), did induce a rapid CNS depression. Otherwise, these chemicals do not have similar actions. However, with respect to the onset and duration of poisoning, they could be divided into two groups.

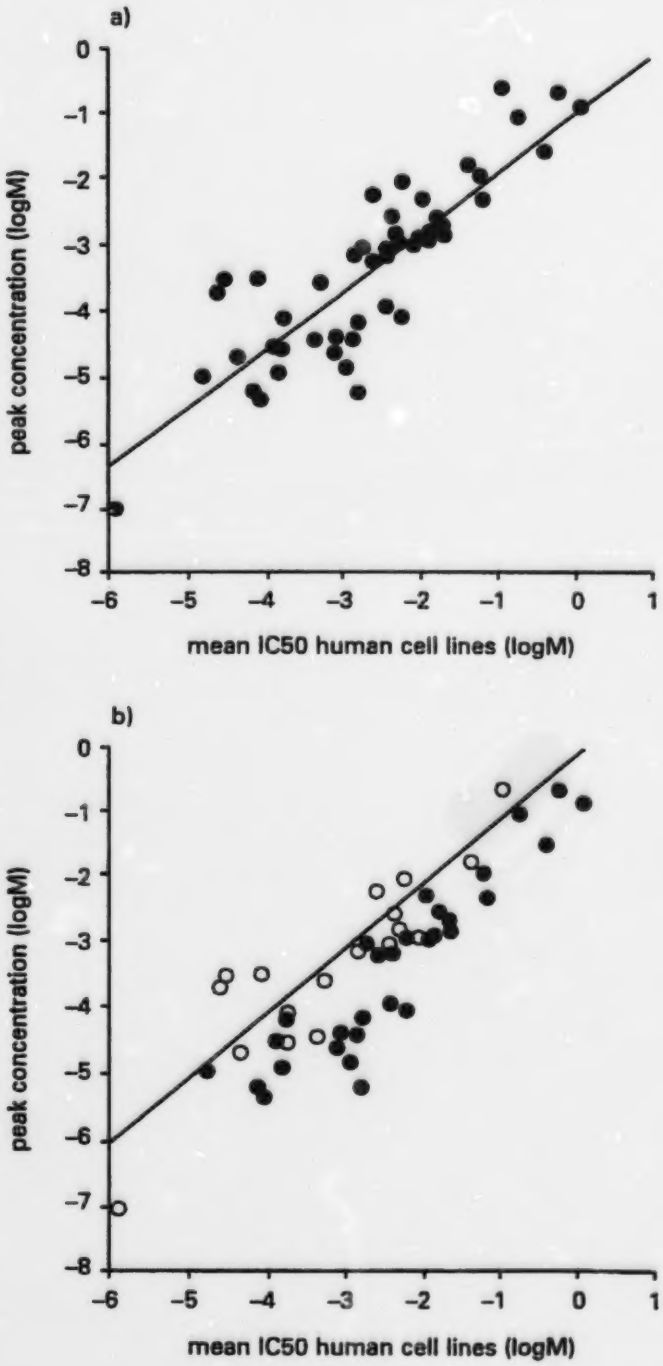
Figure 5: Mean IC50 values (logM) for the 50 MEIC reference chemicals from ten 24-hour human cell line assays (methods 1–10) plotted against post-mortem human lethal blood concentrations for the same chemicals



The forensic lethal concentration values derive from Table VIII of Part V (9). The average IC50 values from tests of chemicals 1–30 by methods 1–10 derive from Table VI of Part III (7). The average IC50 values from chemicals 31–50 tested by methods 1–10 have not been tabulated separately in the preceding papers, but individual IC50 values from tests of chemicals 31–50 by methods 1–10 can be found in Table III of Part IV (9).

$y = 0.885x - 1.162$; $R^2 = 0.711$.

Figure 6: Mean IC50 values (logM) for the 50 MEIC reference chemicals from ten 24-hour human cell line assays (methods 1–10) plotted against peak concentrations from approximate LC50 curves for the same chemicals



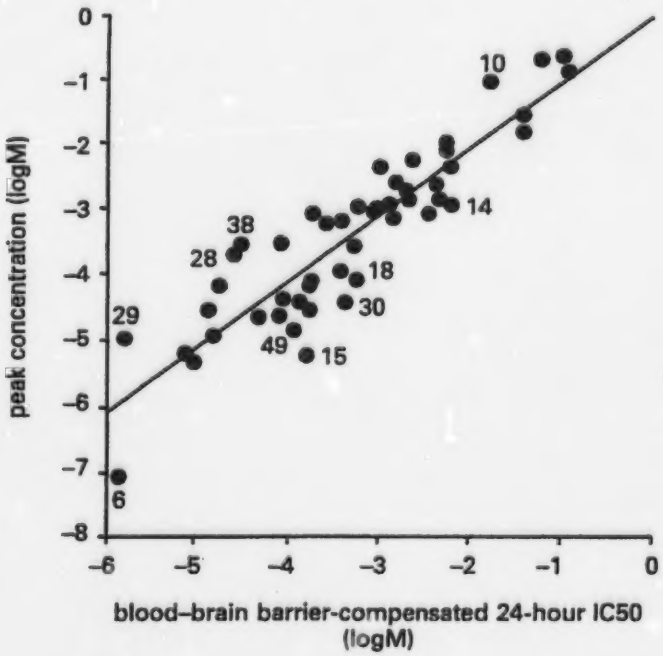
The lethal peak concentrations derive from Table VIII of Part V (9). The average IC50 values from tests of chemicals 1–30 by methods 1–10 derive from Table VI of Part III (7). The average IC50 values from chemicals 31–50 tested by methods 1–10 have not been tabulated separately in the preceding papers, but individual IC50 values from tests of chemicals 31–50 by methods 1–10 can be found in Table III of Part IV (8).

a) Mean 24-hour cell line IC50 (logM) against lethal peak concentration. $y = 0.906x - 0.979$; $R^2 = 0.741$.

b) Datapoints have been used to indicate chemicals with free passage across the blood–brain barrier (●) and chemicals with restricted passage across this barrier (○). A 1:1 line has been inserted instead of the regression line of the preceding figure.

The first group contains seven chemicals which induce rapid lethal symptoms at the time for the peak concentrations, i.e. digoxin, sodium chloride, sodium fluoride, barium nitrate, verapamil hydrochloride, quinidine sulphate and sodium oxalate (chemicals 6, 13, 14, 31, 37, 40, 43 and 46). These chemicals have known (chemicals 6, 13, 31 and 40) or hypothetical (chemicals 14, 37, 43 and 46) lethal actions on various cellular functions, mainly confined to non-CNS organs. Digoxin, verapamil hydrochloride and quinidine sulphate disturb heart conduction and contractility. Barium nitrate is, hypothetically, a neuromuscular blocker. Sodium chloride leads to brain damage and CNS

Figure 7: Mean IC50 values (logM) from tests in ten 24-hour human cell line assays (methods 1–10) of 18 MEIC reference chemicals with restricted passage across the blood–brain barrier and average IC50 values divided by a factor of ten from tests in ten 24-hour human cell line assays (methods 1–10) of 32 MEIC reference chemicals with a free passage across the blood–brain barrier, plotted against peak concentrations from approximate LC50 curves for the same chemicals



The total set of 50 average IC50 values treated in this way were called blood–brain barrier-compensated 24-hour cell line IC50 values (see text). The lethal peak concentrations derive from Table VIII of Part V (9). The average IC50 values from tests of chemicals 1–30 by methods 1–10 derive from Table VI of Part III (7). The average IC50 values from chemicals 31–50 tests by methods 1–10 have not been tabulated separately in the preceding papers, but individual IC50 values from tests of chemicals 31–50 by methods 1–10 can be found in Table III of Part IV (8).

$y = 1.002 - 0.082$; $R^2 = 0.822$.

depression by osmosis without crossing the blood–brain barrier, and sodium fluoride and sodium oxalate also cause CNS-depression through a possibly indirect mechanism, namely calcium depletion. An eighth chemical can be added to this group, i.e. warfarin (chemical 31), which induces bleeding after 36–48 hours by interfering with liver enzymes. Little is known about single overdoses of warfarin, but a delayed action from a peak concentration cannot be excluded. There are few histopathological lesions in organs which result from exposure to this group of eight chemicals.

The second group contains ten chemicals which have a relatively slow (48 hours to several days) effect on several parenchymatous non-CNS organs, sometimes leading to multisystem failure. These chemicals are acetylsalicylic acid, iron (II) sulphate, 2,4-dichlorophenoxyacetic acid, lithium sulphate, arsenic trioxide, copper (II) sulphate, mercury (II) chloride, thallium sulphate, hexachlorophene and pentachlorophenol (chemicals 2, 3, 16, 20, 26–28, 30, 38 and 39). Often, there is extensive histopathological injury to cells. Many of the chemicals are considered to be general cellular poisons (chemicals 2, 3, 16, 26–28, 30, 38 and 39). Thallium sulphate (chemical 30) has delayed effects for up to 3 weeks.

As a second measure, the main lethal action of the 32 chemicals considered to cross the blood–brain barrier freely, was summarised from Table IX of Part V (9). Twenty-two of these chemicals, i.e. diazepam, ethylene glycol, methanol, ethanol, isopropanol, 1,1,1-trichloroethane, phenol, xylene, nicotine, potassium cyanide, dextropropoxyphene hydrochloride, propranolol hydrochloride, phenobarbital, lindane, chloroform, carbon tetrachloride, dichloromethane, orphenadrine hydrochloride, diphenylhydantoin, chloramphenicol, amphetamine sulphate and caffeine (chemicals 4, 7–12, 17–19, 22–24, 32–34, 36, 42, 44, 45, 47 and 48), are usually lethal in humans via a non-specific depression of the CNS, sometimes preceded by a CNS excitation, and very often accompanied by heart arrhythmia and circulatory failure. The CNS depression is usually rapidly induced (minutes/24 hours) at the peak in the blood concentration curve (see Table V of Part V). Five of the chemicals, i.e. amitriptyline hydrochloride, thioridazine hydrochloride, chloroquine phosphate, atropine sulphate and potassium chloride (chemicals 5, 29, 41, 49 and 50) induce CNS depression, but are considered to have heart arrhythmia as their main lethal action. Two of the chemicals, i.e. theophylline and isoniazid (chemicals 21 and 35), seem to have CNS excitation as their main lethal action.

Of the 29 chemicals which induce CNS excitation/depression, 23 chemicals induce lethal loss of function of the CNS without causing any morphological injury to brain cells, as judged by histopathology studies (chemicals 4, 5, 7, 9, 10, 12, 18, 21–24, 29, 32, 33, 35, 36, 41, 42, 44, 45 and 48–50). Another typical trait of CNS-depressant chemicals is that their mechanism of action on CNS is unknown or hypothetical. This applies to 24 of the 29 chemicals, i.e. chemicals 4, 5, 7–11, 17, 21–24, 29, 32–36, 41, 42, 44, 45, 47 and 48. Of the remaining five chemicals with known mechanisms, three are considered to have a general cytotoxic action (chemicals 12, 19 and 50), while nicotine and atropine sulphate (chemicals 18 and 49) have specific, non-cellular effects on CNS transmission (Table IX of Part V). Two CNS depressants, i.e. ethylene glycol and methanol (chemicals 7 and 8), may kill survivors of the depression via the later effects of more-toxic metabolites (glycolate and formic acid) in the brain and in other organs.

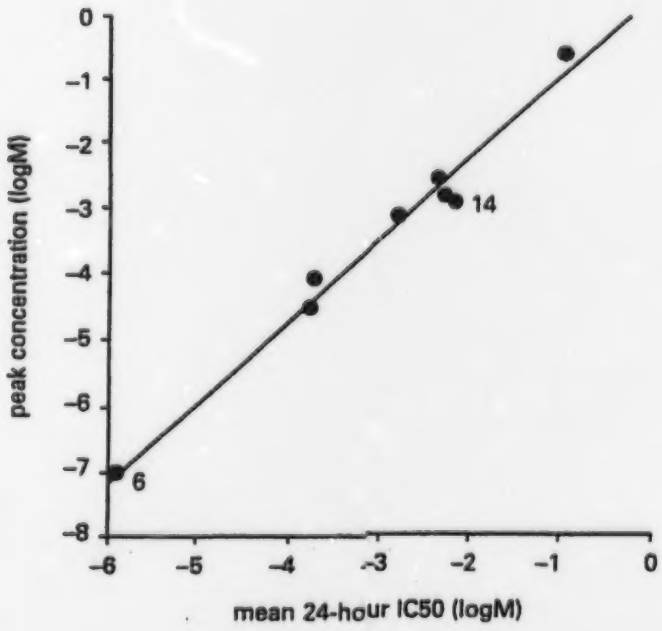
Three of the 32 chemicals that cross the blood–brain barrier do not have CNS excitation/depression (including heart arrhythmia and circulatory failure) as their main lethal action. Malathion (chemical 15) leads to a cholinergic crisis and concomitant respiratory failure. Paracetamol and paraquat (chemicals 1 and 25) may be lethal via a rapid CNS depression at peak concentrations, but usually kill humans (survivors of the CNS depression) by later organ-specific effects caused by the accumulation of intracellular metabolites in the liver or lung, respectively.

As a third measure, the average IC50 values for the 24-hour human cell lines were compared with lethal blood concentrations for each of three groups of chemicals with common traits in their toxic actions, i.e. fast-acting chemicals with a restricted CNS access, slow-acting chemicals with a restricted CNS access, and chemicals with free access to the CNS. All three human lethal concentrations were compared with the *in vitro* IC50 values to assess whether

chemicals with a peak concentration action would be better modelled by peak concentration comparisons than comparisons with the generally lower (untimed) clinical and forensic lethal concentrations, and whether chemicals with a slower action would be comparatively better modelled with clinical and forensic lethal concentrations than with peak concentrations. The correlative findings are presented in Figures 8–11 and Table V.

As a fourth measure, the correlative results for each group of chemicals (Figures 8–11) were compared with the knowledge of the common toxic effects and mechanisms (Table IX of Part V [9]) presented above. After combining the third and fourth measures, the relevance of the *in vitro* results was evaluated. The cytotoxicity results were considered relevant if the good prediction by the IC50 values of the human lethal concentrations could be shown to be consistent with the toxicological knowledge for the compared group of chemicals (or for individual chemicals). Correspondingly, *in vitro* toxicities were judged to be irrelevant if poor correla-

Figure 8: Mean IC50 values (logM) from tests in ten 24-hour human cell line assays (methods 1–10) of a group of eight chemicals with a rapid lethal action and restricted passage across the blood–brain barrier, plotted against peak concentrations from approximate LC50 curves for the same chemicals



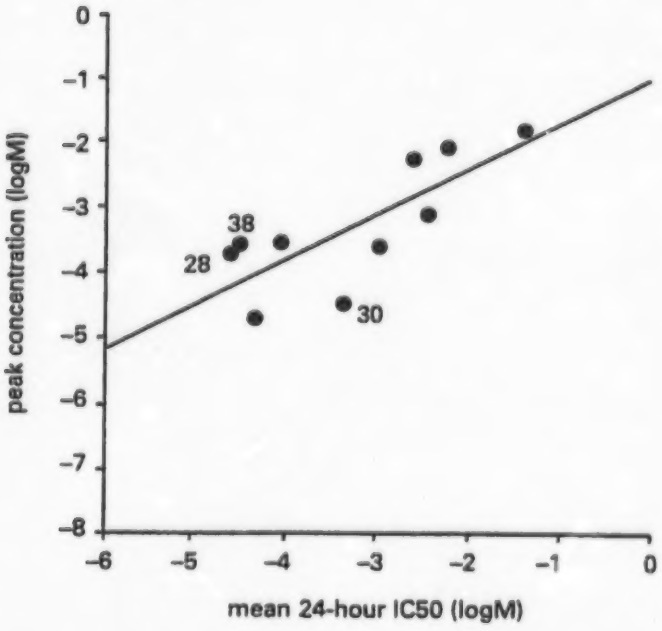
The lethal peak concentrations derive from Table VIII of Part V (9). The average IC50 values from tests of chemicals 1–30 by methods 1–10 derive from Table VI of Part III (7). The average IC50 values from chemicals 31–50 tested by methods 1–10 have not been tabulated separately in the preceding papers, but individual IC50 values from tests of chemicals 31–50 by methods 1–10 can be found in Table III of Part IV (8).

$y = 1.230x + 0.213$; $R^2 = 0.978$.

tions occurred, notably if this was confirmed by mechanistic knowledge.

In Figure 8, the 24-hour average IC50 values with human cell lines for the eight fast-acting chemicals with restricted access to the CNS (see above) are plotted against human lethal peak concentrations. No chemical is a clear outlier in this plot, probably as a result of the steep regression line. The significance of sodium fluoride (chemical 14) as an outlier is in doubt, due to the many potential methodological errors in the study (see above). However, if a less-steep 1:1 line (not shown) is used to judge outlier chemicals, digoxin (chemical 6) will be a clear outlier (more than one log-step). The very good correlation between the IC50 values and the peak concentrations for these chemicals (R^2 = 0.98, slope 1.23) suggests that the 24-hour human cell line cytotoxicity is relevant for the human lethal action for most chemicals, except digoxin. It can be seen in Table V that clinical and forensic lethal concentrations are less well predicted (R^2 = 0.94 and 0.93, respectively), which suggests a lethal action at the initial peak

Figure 9: Mean IC50 values (logM) from tests in ten 24-hour human cell line assays (methods 1–10) of a group of ten chemicals with a slow lethal action and restricted passage across the blood–brain barrier, plotted against peak concentrations from approximate LC50 curves for the same chemicals



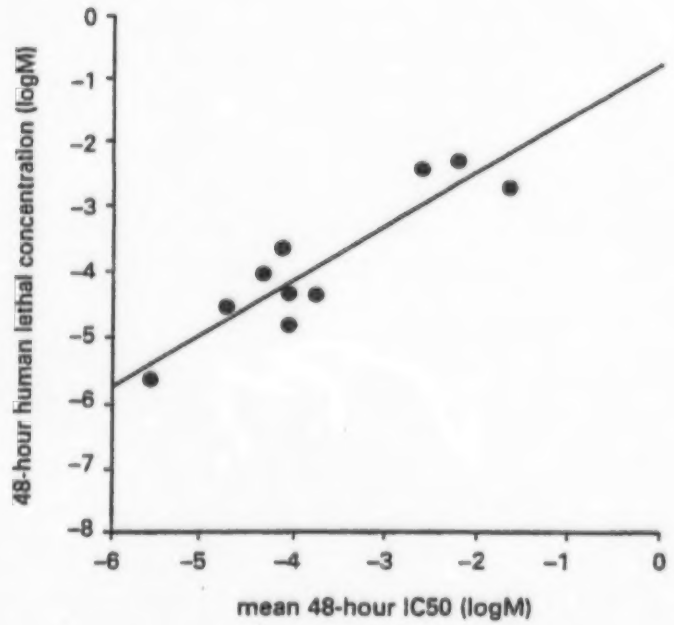
The lethal peak concentrations derive from Table VIII of Part V (9). The average IC50 values from tests of chemicals 1–30 by methods 1–10 derive from Table VI of Part III (7). The average IC50 values from chemicals 31–50 tested by methods 1–10 have not been tabulated separately in the preceding papers, but individual IC50 values from tests of chemicals 31–50 by methods 1–10 can be found in Table III of Part IV (8).

$y = 0.665x - 1.106$; $R^2 = 0.599$.

concentrations, rather than at the lower post-peak concentrations.

The correlative findings are supported by knowledge of the toxicities of these chemicals. The rapid onset of lethal symptoms for seven of the chemicals also indicates a direct action at the peak concentrations. The effect of warfarin may be a delayed result of an action at the peak concentration. The known direct lethal actions of the chemicals on key cellular functions in non-CNS organs (the indirect actions on CNS cells by osmosis and hypothetical calcium depletion not being considered) are consistent with the correlative findings. The focused and functional type of poisoning, as well as the absence of histopathological lesions in target organs, can be explained by fast lethal action by peak concentrations. Organs will not be exposed to a 50% cytotoxic concentration for long enough (24 hours–48 hours) to produce overt cell damage. The outlying behaviour (as judged by the 1:1 line) of digoxin may be explained by a specific action on the Na/K

Figure 10: Mean IC50 values (logM) from tests in three 48-hour human cell line assays (methods 11–13, Table I of Part IV) of the group of ten slow-acting chemicals with restricted passage across the blood–brain barrier, plotted against human lethal concentrations of the same chemicals after exposure for 48 hours, derived from approximate LC50 curves



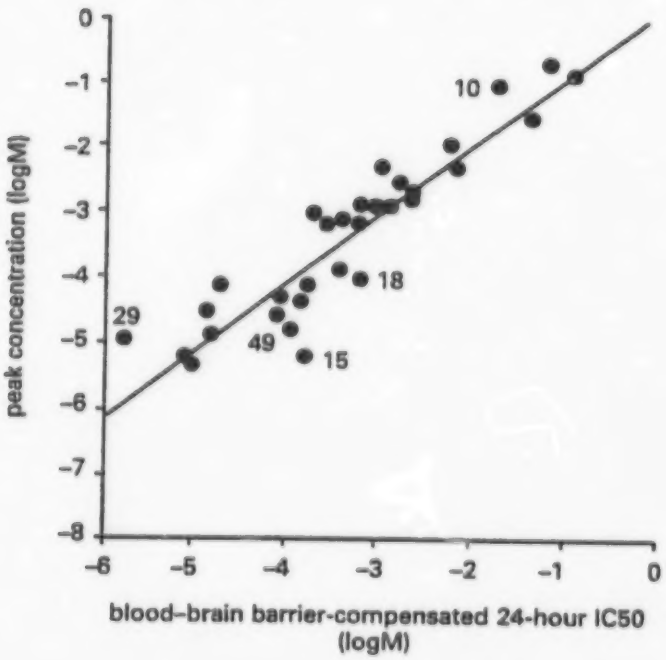
The 48-hour LC values derive from the Internet Edition of MEIC Monographs 1–50 (14). The average IC50 values have not been published separately in the preceding articles (6–8). IC50 values for individual methods can be found in references 6–8, namely in Table I of Part II (chemicals 1–30 tested by method 13 [old number 10]), Table III of Part III (chemicals 1–30 tested by methods 11 and 12 [old numbers 11A and 12]), and Table III of Part IV (chemicals 31–50 tested by methods 11–13).

$y = 0.804x - 0.875$; $R^2 = 0.820$.

ATPase on heart cells. Some of the human cell lines do not have this target enzyme at high enough levels to be adequately sensitive to digoxin, which may lead to underprediction and a reduced relevance of the average 24-hour human cell line IC50 values (see Discussion). To sum up, the 24-hour cytotoxicity to human cell lines seems to be relevant to the human acute lethal action for most of the chemicals, but not for digoxin.

In Figure 9, the 24-hour average IC50 values in human cell lines for the ten slow-acting chemicals with restricted access to the CNS (see above) have been plotted against the human peak concentrations. Thallium sulphate (chemical 30) is a clear outlier. Due to the flat regression line, mercury (II) chloride and hexachlorophene (chemicals 28 and 38) are not outliers. If the latter two chemicals are considered on the basis of a steeper 1:1 line (not shown), they will be moderate (less than one log step) outliers. The relatively good correlation between IC50 values and peak concentrations (R^2 = 0.60, slope

Figure 11: Mean IC50 values (logM) divided by a factor of ten from tests in ten 24-hour human cell line assays (methods 1–10) of a group of 32 MEIC reference chemicals with free passage across the blood–brain barrier, plotted against peak concentrations from approximate LC50 curves for the same chemicals



The lethal peak concentrations derive from Table VIII of Part V (9). The average IC50 values from tests of chemicals 1–30 by methods 1–10 derive from Table VI of Part III (7). The average IC50 values from chemicals 31–50 tested by methods 1–10 have not been tabulated separately in the preceding papers, but individual IC50 values from tests of chemicals 31–50 by methods 1–10 can be found in Table III of Part IV (8).

$y = 1.029x + 0.073$; $R^2 = 0.850$.

0.67) for these ten chemicals certainly suggests some relevance of the IC50 values for the human lethal action of the chemicals, except for thallium sulphate. However, it is difficult to explain the low *in vitro/in vivo* correlation (R^2 = 0.60) for this group, compared to the very light correlation for the previous group (R^2 = 0.98).

One explanation could be that the peak concentration is irrelevant for the slow action of these chemicals. Hypothetically, the relevance of the cytotoxicity of these chemicals would be assessed better by a comparison of the *in vitro* and *in vivo* concentrations from longer exposure times, corresponding to the delayed effects of the substances.

To test this hypothesis, average IC50 values for the ten slow-acting chemicals from three 48-hour tests with human cell lines (methods 11–13 [8]) found in Table I of Part II (6), Table III of Part III (7) and Table III of Part IV (8), were plotted against 48-hour concentrations from approximate LC50 curves over time in humans (14). The results are shown in Figure 10. The plot is not fully comparable with the other plots of this analysis (Figures 4–9 and 11), because of the less well established base of only three tests for the average 48-hour IC50 value, compared with the ten tests for the average 24-hour IC50 value.

The correlation in Figure 10 (R^2 = 0.82, slope 0.80) is much better than the correlation between the 24-hour IC50 values and peak concentrations (R^2 = 0.60), which suggests that the hypothesis is correct. There are no clear outliers. This result is correlative evidence for the relevance of 48-hour human cell line cytotoxicity for the human lethal action of most of the chemicals in the group.

The results shown in Figure 10 may also be used to analyse further the results shown in Figure 9. The correlation (R^2 = 0.60) between the 24-hour IC50 values and the peak concentrations in Figure 9 provides evidence of the rather indirect relevance of the 24-hour *in vitro* cytotoxicity for the lethal action of the chemicals at the peak concentrations. The positive correlation obtained is a function of the similarity between the 24-

Table V: Prediction of human lethal concentrations by 24-hour human cell line assays for groups of chemicals with different actions, when compared with prediction for all 50 chemicals[a]

	Clinical lethal concentrations		Forensic lethal concentrations		Peaks from LC50 curves	
Group of chemicals	**Slope**	**R^2**	**Slope**	**R^2**	**Slope**	**R^2**
Eight chemicals with fast action, and restricted entry to central nervous system	1.29	0.94	1.29	0.93	1.23	0.98
Ten chemicals with slow action, and restricted entry to central nervous system	0.74	0.55	0.66	0.59	0.67	0.60
32 chemicals with free entry to central nervous system[a]	0.95	0.70	0.92	0.75	1.03	0.85
All 50 chemicals[a]	0.97	0.71	0.94	0.73	1.00	0.82

[a]*Mean IC50 values of chemicals freely entering the central nervous system have been divided by ten.*

hour and 48-hour cytotoxicities, as well as between the peak concentrations and the 48-hour blood concentrations for the majority of these chemicals, with a long biological half-life (see reference 14, and Table V of Part V [9]). Hence, the spread of data-points in the plot is caused by the comparison of irrelevant parameters.

The correlative findings in Figure 10 are supported by substantial knowledge of the toxicities of the ten slow-acting chemicals (see above), with widespread lethal effects on many non-CNS organs. Firstly, the slow lethal action of these chemicals explains that *in vitro* and *in vivo* concentrations for 48-hour exposure correlate better than concentrations for 24-hour or shorter exposures. Since only sustained action provokes lethal injury, short exposure of the non-CNS organs to peak concentrations exceeding the 24-hour/48-hour IC50 value may be tolerated. Secondly, the known or hypothetical human lethal action mechanisms for these chemicals are cytotoxicity. Lastly, the widespread histopathological injury caused by these chemicals also indicates basal cytotoxicity, analogous to conditions *in vitro*. To summarise results, the 48-hour lethal action to human cell lines seems to be relevant for the human lethal action of most chemicals in this group.

In Figure 11, the blood–brain barrier-compensated average 24-hour IC50 values for human cell lines for the 32 chemicals with free entry to the CNS (see above) are plotted against human lethal peak concentrations. There is a good correlation between these data (R^2 = 0.85, slope 1.03). One chemical is a clear (more than one log step) outlier in this plot, i.e. malathion (chemical 15). Other minor outliers are isopropanol, nicotine, thioridazine hydrochloride and atropine sulphate (chemicals 10, 18, 29 and 49). Note that the near 1:1 slope of the regression line in this plot coincidently makes these minor outliers comparable to the clear outliers previously discussed. It can be seen from Table V that peak concentrations are much better predicted than the (untimed, often later) clinical and forensic lethal concentrations (R^2 = 0.70 and 0.75, respectively), which indicates a rapid lethal action.

The improved predictions through the blood–brain barrier compensation of IC50 values for these chemicals (Figures 6 and 7), as well as the good correlation between IC50 values and peak concentrations in Figure 11, suggest the relevance of the 24-hour *in vitro* toxicity to the human lethal action for most of the chemicals. Specifically, there is correlative evidence for a human lethal action by peak concentrations containing a fraction (around one tenth) of the 24-hour IC50 value. The correlative results highlight the action of malathion as an exception, and the actions of isopropanol, nicotine, thioridazine and atropine as possible exceptions.

The correlative findings are strongly supported by the toxicological data in Table IX of Part V (9). Firstly, 29 of the 32 chemicals actually cause a CNS excitation/depression at the time of the peak concentration. Secondly, for most of the chemicals there is no histopathology in the CNS, indicating a loss of cell functions caused by a fraction of the IC50, after a short exposure time. Thirdly, most of these 29 chemicals either have unknown mechanisms or known cellular mechanisms of toxicity. Only three of the chemicals (malathion, nicotine and atropine sulphate [chemicals 15, 18 and 49]) are known to kill humans via non-cytotoxic effects, and all these three chemicals are seen to be clear or minor outliers in Figure 7. Fourthly, the correlative findings are not contradicted by the well-known concomitant initial effects on heart and circulation of many of the CNS-depressive chemicals. Such symptoms may be caused by the initial, parallel effects of basal cytotoxicity to the two other organs with recognised short-term vital functions, i.e. heart conduction tissue and capillaries. Lastly, these conclusions are not contradicted by the known slower lethal effects on non-CNS organs in survivors of CNS depression, after exposure to some chemicals, such as paracetamol, ethylene glycol, methanol and paraquat (chemicals 1, 7, 8 and 25). Such effects might be the result of post-peak accumulation of these chemicals in the target organs (for paracetamol and paraquat), or later action by more-toxic metabolites (for ethylene glycol and methanol), at concentrations near to the CNS-depressive, lethal concentration curve.

To sum up, 24-hour human cell line toxicity seems to be relevant to human lethal action for most of the 32 chemicals. Cell line toxicity seems to be irrelevant for the human lethal action of some chemicals in the group, namely the acute actions of malathion, nicotine and atropine sulphate, and the most

common, slow lethal effects of paracetamol and paraquat. The 24-hour human cell line toxicity may also be irrelevant for potassium cyanide, in spite of the correlative evidence of a similarity between IC50 values and lethal concentrations. This chemical inhibits cytochrome oxidase, and hence cellular respiration (9). Most, if not all, cells of the ten 24-hour cell line systems have anaerobic culture conditions, and therefore should not be adequately sensitive to potassium cyanide. Instead, there may be another explanation for the similarity of IC50 and lethal concentration, i.e. that the lethal concentration value is derived from whole-blood concentrations, which for potassium cyanide probably merely represents organ accumulation in blood cells, corresponding to lower real serum values, as indicated in Part V (9). The relevance of the *in vitro* toxicity to the actions of isopropanol and thioridazine hydrochloride is unclear; possibly the positions of these minor outliers in Figure 11 are artifacts caused by the potentially large margin of error of the data being compared (see above). Thioridazine hydrochloride may, for instance, be an outlier because of a comparatively low IC50 value due to the high protein binding of the chemical.

The above analyses permit an interpretation of the correlation between blood–brain barrier-compensated 24-hour IC50 values for human cell lines and the human lethal peak concentrations of all 50 chemicals, presented in Figure 7 and Table V. The good correlation (R^2 = 0.82, slope 1.0) is a composite of the correlations involving peak concentrations for each of the three groups in Figures 8–10 (R^2 = 0.98, 0.60 and 0.85, respectively). As such, the correlation incorporates all the outliers found in the separate studies, i.e. the three major outliers, digoxin, malathion and thallium sulphate (chemicals 6, 15 and 30), and seven minor outliers (chemicals 10, 14, 18, 28, 29, 38 and 49). The results of this plot are interesting from a practical point of view, because it suggests a potential for good prediction of human peak concentrations by a small battery of 24-hour human cell line assays combined with knowledge of, or tests on, chemicals crossing the blood–brain barrier. However, as a correlative indicator of the relevance of *in vitro* tests for human lethal action of chemicals, the plot is misleading, because it includes the suboptimal 24-hour cell test predictions of human peak concentrations for the ten slow-acting chemicals (Figure 9).

To summarise the results shown in Figures 8, 10 and 11, the high correlations between *in vitro* IC50 values (24-hour or 48-hour) and the human lethal concentrations (peaks and 48-hour) for the three groups of chemicals (R^2 = 0.98, slope 1.23; R^2 = 0.82, slope 0.80; and R^2 = 0.85, slope 1.03, respectively) suggest a relevance of human cell line toxicity for most chemicals. Exceptions are the two clear outliers, digoxin and malathion, and probably the two minor outliers, nicotine and atropine sulphate, which are all known to poison humans via a highly receptor-specific action, not always found in cell line cultures. Other minor outliers in the plots have been sodium fluoride, thallium sulphate, isopropanol and thioridazine hydrochloride. The outlier position of thallium sulphate might be explained by its extremely slow action, not measured well in the 48-hour cell line tests. It is difficult to judge the relevance of the *in vitro* results for the three remaining outliers. These chemicals all have CNS-depressive and/or cytotoxic effects in the body, reminiscent of the majority of the chemicals. The outlier positions could be insignificant "false poor correlations", caused by poor raw data or other methodological errors. Another difficulty is to properly judge the good correlations for paracetamol and paraquat, which are compatible with their CNS-depressive effects, but not with their slower, main lethal effects on the liver and lung, respectively. The results for paracetamol and paraquat could be "false good correlations". Likewise, the results for potassium cyanide could also be a "false good correlation" (see above). Note that other chemicals in the study could have made "false good correlations", which were impossible to detect due to poor human toxicity data.

In addition to the margins of error of the compared reference data for some chemicals already discussed, another factor can be suspected to lead to "false bad correlations" or "false good correlations", i.e. a suboptimal blood–brain barrier compensation factor. This factor of ten represents preliminary "hard" modelling. Initially, the choice of this factor was guided by the results from previous studies (10, 11, 13). Another reason to use this factor was the good fit of the total

data in Figure 7 as a result of this algorithm. At that stage of the analysis, known mechanisms and effects of chemicals could not be taken into consideration. Otherwise the analysis would be merely circular reasoning. However, in retrospect (after identification of significant outliers), it is possible to assess how this factor was chosen and also to analyse how a suboptimal choice of the blood–brain barrier-compensating factor may lead to "false bad correlations" or "false good correlations".

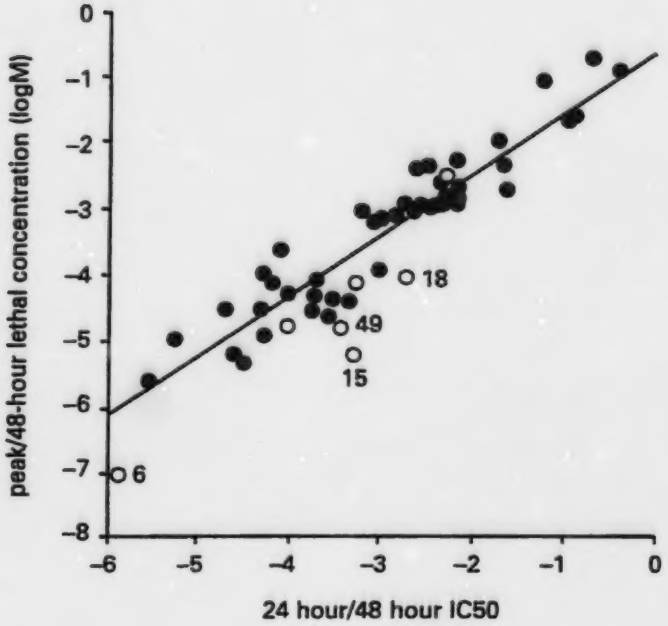
The factor of ten was chosen to adapt the correlation for the 32 chemicals passing across the blood–brain barrier to the correlation for the other chemicals. The plot for the 32 chemicals included significant *in vitro* underpredictions of the human toxicity of malathion and atropine sulphate. The plot for the remaining chemicals included clear *in vitro* overpredictions of human toxicity for mercury (II) chloride and hexachlorophene (see Figures 6a and 7). The over-shooting adaption of the underpredicted chemicals to the two overpredicted chemicals seems to have resulted in a suboptimal, excessively large factor. An excessively large blood–brain barrier factor would, in turn, be expected to lead to "false bad correlations", i.e. *in vitro* results for chemicals belonging to the CNS group which seem to overpredict human toxicity only because they have been reduced too much. It is notable that the minor outliers, thioridazine hydrochloride and isopropanol, are both CNS depressants and have *in vitro* toxicities which overpredict human toxicity. Corresponding "false good correlations" could have been obtained by this over-shoot adaption for chemicals in the CNS group, with *in vitro* results which, in reality, underpredict human toxicity. Paracetamol and paraquat are candidates for such "false good correlations".

Multivariate PLS analysis and other soft modelling methods will be used to select batteries at later stages of the MEIC evaluation. Also, a refined "individual" correlative/mechanistic analysis for each chemical is planned, comparing the whole approximative LC50 curve with *in vitro* toxicities from different time-points, taking more factors into consideration. The latter study is especially suited to discriminating between significant and insignificant correlations.

These forthcoming studies do not exclude a final step of simple soft modelling in the present analysis, to explore whether a preliminary optimisation of the blood–brain barrier factor could improve discrimination between significant and insignificant minor outliers (nicotine, atropine sulphate, isopropanol and thioridazine hydrochloride). The study was also performed to test whether the correlations for paracetamol, paraquat and thallium sulphate (the three chemicals of the study with some inconsistency between correlative results and actions) may have been "false good correlations". The study was performed in several steps. The final steps (4 and 5) are presented in Figure 12.

1. The data for the first two groups with restricted passage across the blood–brain barrier (i.e. the data in Figures 8 and 10) were plotted in the same graph, except for the data of the clear outliers, digoxin and thallium sulphate (chemicals 6 and 30). Note that the plot is a composite of 24-hour and 48-hour data, which is against the rules of linear regression analysis.
2. The data for the group of chemicals with free passage across the blood–brain barrier (i.e. data in Figure 11) were plotted on another graph, except for the data of the clear outliers, malathion, nicotine and atropine sulphate (chemicals 15, 18, and 49), and the data for paracetamol and paraquat (chemicals 1 and 25).
3. A comparison of the two equally scaled graphs indicated an optimal correlation of all the data, this time with use of a factor of a half log step for the blood–brain barrier compensation.
4. The IC50 values for the 27 remaining chemicals in the third group were reduced by a half log step (i.e. divided by 3.16). The new data were plotted together with the composite data of the other two groups (see step 1), resulting in the 43-chemical graph in Figure 12 (peak lethal concentration/48-hours).
5. Finally, the data for the seven withdrawn chemicals were merely positioned on the graph, after a half log step reduction of the IC50 values for chemicals 1, 15, 18, 25 and 49, but not for chemicals 6 and 30. Hence, the seven added chemicals are not included in the correlation (regression formula and regression line) for the other

Figure 12: Mean 24-hour/48-hour IC50 values (logM) for 43 MEIC reference chemicals plotted against human lethal peak concentrations/48-hour lethal concentrations



The regression line and regression formula derive from this original plot for 43 chemicals. Chemicals in this group with free passage across the blood–brain barrier have been divided by 3.16. Also, data points for seven clear or suspected outlier chemicals (○) from preceding plots (Figures 7 and 11), i.e. digoxin, malathion, nicotine, atropine sulphate, thallium sulphate, paracetamol and paraquat (chemicals 1, 6, 15, 18, 25, 30 and 49) have then been positioned in the plot for comparison.

$y = 0.913x - 0.614$; $R^2 = 0.892$.

The steps employed to make the composite, 50-chemical plot were as follows: a) the data for the first two groups with restricted passage across the blood–brain barrier (i.e. the data in Figures 8 and 10) were plotted in the same graph, except for the data of the clear outlier digoxin (chemical 6), and thallium sulphate (chemical 30). Note that the plot is a composite of 24-hour and 48-hour data, which is against the rules of linear regression analysis; b) the data for the group of chemicals with free passage across the blood–brain barrier (i.e. data in Figure 11) were plotted in another graph, except for the data of the clear outliers malathion, nicotine and atropine (chemicals 15, 18 and 49), and the data for paracetamol and paraquat (chemicals 1 and 25); c) a comparison of the two equally scaled graphs indicated an optimal correlation of all data, this time with use of a factor of 0.5 log step for the blood–brain barrier compensation; d) the IC50 values for the 27 remaining chemicals in the third group were reduced with 0.5 log step (divided by 3.16). The new data for the 27 chemicals were plotted together with the composite data of the other two groups (see step a, resulting in the 43-chemical plot [●]); e) finally, the data for the seven withdrawn chemicals were positioned in the graph (○), after a 0.5 log step reduction of the IC50 values for chemicals 1, 15, 18, 25 and 49, but not for chemicals 6 and 30. Hence, the seven added chemicals are not included in the correlation for the other 43 chemicals.

43 chemicals.

The plot of peak lethal concentration/48-hours in Figure 12 includes all the chemicals which have correlated well in Figures 8, 10 and 11 (except for paracetamol, paraquat and thallium sulphate) and all the minor outliers with no positive evidence of non-cellular toxicity. The correlation (R^2 = 0.89, slope 0.91) is good, but difficult to interpret properly, due to the mixed raw data on each axis. However, all four mechanistically understood outliers, i.e. digoxin, malathion, nicotine and atropine sulphate (chemicals 6, 15, 18 and 49), are now more clearly separated from the other chemicals, compared with what is seen in previous plots (for example, Figure 7). The former minor outliers, isopropanol and thioridazine hydrochloride, are not prominent. Also, the chemicals with a suspected "false good prediction" are not outliers, which suggests that they have significant correlative predictions in the previous comparisons (Figures 8, 10 and 11). Finally, the results suggest that a factor of ten is too large for blood–brain barrier compensation purposes. This excessively large factor probably contributed to the outlier positions of thioridazine and isopropanol in the previous hard modelling.

The *in vitro/in vivo* correlations found can be used to test the first hypotheses of the basal cytotoxicity concept, as outlined in the Introduction (1, 11).

1. The central hypothesis claims that the majority of non-selected chemicals cause acute lethal toxicity in humans via basal cytotoxicity, while a minority of chemicals have an organ-specific cellular action or a non-cellular target action. A second hypothesis claims that basal cytotoxicity can be tested with undifferentiated cell lines. The high correlations between cell toxicity and human lethal concentrations, as well as the few outlier chemicals found in this study, strongly supported these hypotheses. Only four out of 50 chemicals (digoxin, malathion, atropine sulphate and nicotine) were clearly pointed out to have specific lethal actions, which could not be attributed to basal cytotoxicity. Even if outliers are defined more liberally, also to include "false good predictions" for paracetamol, potassium cyanide and paraquat, as well as some moderate outlier chemicals with unknown mechanisms, at least 80% of the chemicals of this study (randomly selected from this point of view) seem to have basal cytotoxicity (= average human cell line toxicity) as the basis for their acute lethal action in humans.
2. According to another basal cytotoxicity hypothesis, non-specific CNS excitation/depression is the obligatory reaction of the body to the basal cytotoxicity of chemicals, provided that the compounds freely cross the blood–brain barrier. Also, an extra vulnerability of brain function (compared to functions of the non-CNS organs) has been assumed, leading to lethal CNS depression from a fraction (hypothetically around one tenth) of the 50% cytotoxic concentration. The first hypothesis is strongly supported by the induction of CNS excitation/depression by 29 of the 32 chemicals which can enter the brain freely (malathion, atropine sulphate and nicotine not counted), and used in this study. The second hypothesis is supported by the improved correlations between IC50 values and peak concentrations for all the chemicals, when a factor of ten was used to adapt the IC50 data (Figures 6 and 7). However, the later soft modelling of all the chemicals, except for the outliers in Figure 12, suggests that the optimal factor may be lower than ten.
3. According to the basal cytotoxicity concept, basal cytotoxicity is a minimal baseline toxicity for all chemicals. When a basally cytotoxic concentration is reached in the body (for a sufficient time for slow-acting chemicals), humans will succumb. Thus, adequate *in vitro* test results should not produce false positive predictions. This hypothesis is verified by the present results. No (one log step) false positive predictions have been found among the 50 chemicals. The two minor outliers in the plots (Figures 7, 8, 10 and 11) with high IC50 values compared with the human concentration (thioridazine hydrochloride and isopropanol), probably resulted from the use of an excessively large blood–brain barrier compensation factor.

Discussion

As outlined in the Introduction, this paper pre-

sents three separate studies with three different aims. In the interests of clarity, the results will be discussed under separate headings.

Prediction by rat and mouse LD50 values

The LD50 test in rodents has a major role in toxicity testing. The advantages and disadvantages of the test methodology and the information obtained have been discussed (15, 16), including severe criticism of various aspects of the test (1, 17). The test has never been formally validated (15). The widespread use of the test has therefore not been based on a documented good performance, but on the lack of better tests.

The results of this study (Figures 1–3 and Table I) will be used in the forthcoming evaluation as a base-line for assessing the relative effectiveness of batteries of *in vitro* tests from the MEIC study. However, since practically no validation of the LD50 test has been performed, the results may be of general interest to all toxicologists.

The similarity of rat and mouse LD50 results, with only three clear outlier chemicals (Figure 1), can be expected, as the two species are relatively closely related. Yet one should not automatically blame species differences for bad correlations. This could also depend on the varying quality and standardisation of the individual tests compared, which cannot be analysed in this study. Separate tests with the same animal species are known to vary considerably (17).

The moderately good correlation between rat LD50 and human acute lethal doses (R^2 = 0.61, slope 0.83), which includes as many as seven clear outliers (see Figure 2), is the result of a combination of four factors, i.e. the large species difference, the poor quality of some of the experiments (see above), the comparison of LD50 values to not-quite comparable human lethal dose values, and the poor quality of some of the lethal dose values. The last two factors were discussed in Part V (9). The species difference is probably the most decisive factor, but the influence of the other factors could confound the results, making it difficult to discuss seriously bad correlations for chemicals in terms of species difference and chemical action.

The somewhat better correlation between mouse LD50 and human acute lethal doses (R^2 = 0.65, slope 0.90), including six clear outliers (see Figure 3), ought to be the result of the same factors as the rat/human correlation. The last three factors could still confound the results, as discussed above.

Some of the most conspicuous bad correlations between rodent LD50 and human lethal dose for individual chemicals in Table I should be commented on, with a focus on chemicals which are outliers in both comparisons. The clear underprediction of the human toxic dose of digoxin by tests in both rodent species is well known (2, 6–8), and can be explained by the relative lack of the target enzyme Na/K ATPase activity in most animal cells when compared with most human cells (18). Thus, digoxin is underpredicted by all the animal cytotoxicity assays shown in Table IV. The clear rodent test underprediction for atropine sulphate suggests that cholinergic transmission, or rather, the vital importance of cholineric transmission, might not be the same in rodents and humans. The action of warfarin as a rodenticide could putatively be connected to the overprediction of the human toxicity in the rodent tests. The authors are not aware of any good mechanistic explanation for the rodent test underprediction for sodium oxalate.

It is interesting to note that both rat and mouse LD50 tests clearly underpredict human toxicity for as many as two of the four chemicals with a specific receptor toxicity, which were also poorly predicted by the human cell cultures, i.e. digoxin and atropine sulphate. One strong argument for the superiority of animal tests over cellular tests has been that animal tests are also capable of testing chemicals with non-cellular toxic mechanisms, thereby being more predictive than cellular assays. The results of this study do not support that argument, but suggest that animal tests may be poor predictors of toxicity for specific receptors or neurotransmission. This could be because neurotransmission and other sophisticated receptor-mediated physiology is much more species-specific than basal cellular physiology. The high variability of human lethal doses of atropine sulphate (Table II of Part V), and the clinical and forensic lethal concentrations for malathion (Tables III and IV of Part V), even suggest a high inter-individual variation of the human toxicity of these chemicals.

The results from this study of 50 non-selected chemicals are probably representa-

tive and may be generalised. To try to confirm this, and to further explore animal LD50 test prediction of human toxicity, the authors plan to further extend the present study to compare rodent LD50 values and human lethal dosage for a larger number of chemicals.

Prediction by 61 in vitro toxicity tests

Tables II–IV display the results of purely correlative comparisons for IC50 values from all the methods in which the full set of 50 MEIC reference chemicals were tested. The evaluation starts with comparisons of individual methods (Table II). Then groups of methods are evaluated, as comparisons of average IC50 values for the group with human concentrations (Table III). Finally, clear outliers from the group correlations are listed in Table IV.

The aim of the evaluation is only to present an overview, leading to gross correlations. In-depth analysis, relating findings to the characteristics of the specific non-human *in vitro* systems, or to chemical effects, or actions in these systems, is outside the scope of this study, i.e. no such data have been compiled in the MEIC *in vivo* toxicity database (9). The scarcity of such toxicity data makes any such analysis difficult.

In Table II, three sets of independently collected lethal concentrations have been compared to the *in vitro* test results. Most *in vitro* test systems seem to predict best the peak concentration, which suggests that these peak concentrations are the most relevant *in vivo* reference data. Judged from the prediction of peak concentrations, the predictions by human cell line tests are best (mean $R^2 = 0.64$). The prediction then goes down as the test organisms get phylogenetically more distant (mean $R^2 = 0.36$ for non-fish ecotoxicological systems). This is as expected, but (similarly to the previous analysis of rodent prediction of human toxicity) one should avoid attributing this only to species differences between the test systems. Many other factors come into play, the most important of which is the *in vitro* system, notably the conditions of exposure. This was discussed in Part II (6).

The most striking feature of the results presented in Table II is the similarity of the predictions given by most tests within each group. This similarity, and the deviating performance of some tests for a variety of reasons (short exposure times, subjectively determined endpoints, etc.), have been analysed thoroughly in Parts II, III and IV (6–8).

Some assays within each group seem to predict toxicity better than others. Of the human cell line tests, method 18 (measuring the protein content of MRC-5 cultures after 6 weeks; 5) has an outstanding prediction ($R^2 = 0.80$, slope 0.78) of acute human toxicity. It is a paradox that a chronic toxicity test aimed at measuring the effects of repeated dosage of chemicals to cells should be able to predict acute toxicity at all. However, when certain features of the test are considered, the result is less surprising. The protein content measured after 6 weeks is probably the result of the initial short-term action of the chemical, and is not changed much by further exposure. due to the development of tolerance of cells (overgrowth in cultures of resistant cells, surviving the initial attack). Hence, the initial events are only slightly modified by the 10 (2×5) repeated doses during the following 5 weeks. All these dosages may increase the precision of the test, as does an increase in the number of repeated tests in any toxicity assay, the average result of which will be more reliable than the results from each separate test or a few repeated tests.

Among the animal cell line tests, method 31 (measuring the protein content of 3T3-L1 mouse cell line cultures after 72 hours; 5), seems to be the most predictive, especially if the number of chemicals tested is considered ($R^2 = 0.64$, slope 0.75). The use of mouse cells may have contributed to the result, since mouse cells have been found to predict human cell line toxicity better than rat cells, in analogy with the comparatively superior mouse prediction of human toxicity (4, 5). Among non-human primary cultures, the rat skeletal muscle assays (methods 39–41) gave the best predictions, the R^2 of 0.64 from tests on 49 chemicals being more impressive than higher R^2 values from tests on fewer chemicals. Among the fish cell cultures, methods 45 and 46 were the best predictors, as judged from both R^2 values (0.60–0.59) and slopes. Finally, the most predictive non-fish ecotoxicological test was method 53, a *Daphnia magna* immobilisation test with exposure for 24 hours, resulting in an R^2 of 0.51.

The very interesting results from the ovalbumin denaturation assay have been discussed previously (8). The good prediction of human cell line cytotoxicity probably supports the good prediction of human toxicity by this test, suggesting protein denaturation as a frequent mechanism of cell line toxicity (8). Follow-up studies of these potentially important results are urgently required.

The study of individual methods, presented in Table II, did not use the blood–brain barrier-compensating factor to achieve increased predictivity, for two reasons. Firstly, the factor of ten could be anticipated to be suboptimal. Secondly, the use of this algorithm may potentially distort the comparisons for specific methods, notably methods that are extra sensitive with respect to CNS injury, such as methods 33 and 41, which measure inhibition of contractility in rat muscle cells (6, 7, 19).

The group prediction in Table III was made only to identify outlier chemicals. It is impossible to present outlier chemicals for each individual method for practical reasons. Steps were taken to make a robust study of outliers. Statistically more-stable group results were used, and criteria were established to make outliers comparable, as well as reliable. Another step taken to provide robustness was to reduce the number of potential outliers by the use of the best possible prediction. Therefore, outlier chemicals for the groups were judged according to the blood–brain barrier-compensated peak prediction. To link this prediction with the prediction of individual tests (Table II), the results in Table III also include simple predictions of the three human lethal concentrations.

The increase (sometimes ten-fold) found in the group prediction of human lethal concentrations, compared to the prediction by individual tests, was expected. The same principle is routinely followed by *in vitro* toxicologists, when several single test results are used to make up a more reliable average value. The selection of relevant outlier chemicals is probably better if they are derived from group correlations, rather than the correlations from individual assays. One must be aware of pitfalls in using collective predictions, however. It will only work if the tests in each group are similar. If very dissimilar tests are put together in one group (for example, non-fish ecotoxicological tests), the effect is the opposite, i.e. the results (correlations, outliers) are obscured by lack of discrimination between the differences of the individual tests.

The general features of the chart of outliers in Table IV can be anticipated from the correlations in Table III. The prediction of human toxicity by the different groups is proportional to the number of outliers. Another feature is a consistency through the whole phylogenetic series of bad correlations for some chemicals, indicating similar prediction by all the systems. Thus, all outlier chemicals in the human cell predictions are also outliers in most other assays.

The results of the correlative/mechanistic analysis of the 24-hour human cell line prediction of human toxicity made in this paper, makes it possible to evaluate the significance of the most conspicious and consistent outliers in Table IV. Thus, the overestimation of human toxicity by nearly all groups of assays for mercury [II] chloride, thioridazine hydrochloride and hexachlorophene (chemicals 28, 29 and 38), is probably caused by the selection of irrelevant human reference data for the comparisons (peak concentrations for mercury [II] chloride and hexachlorophene) and, possibly, too-large a blood–brain barrier compensation factor for thioridazine hydrochloride. The three chemicals also have a high blood protein binding capacity, which may lead to overprediction of toxicity in *in vitro* systems with low protein media, such as the non-fish ecotoxicological tests. Likewise, the underestimation of human toxicity for thallium sulphate (chemical 30) by some groups of assays is probably caused by the choice of irrelevant reference values, i.e. peak concentrations.

On the other hand, the underestimation of the human toxicity of digoxin, malathion and atropine sulphate (chemicals 6, 15 and 49) by most groups of assays, as well as the underestimation for nicotine (chemical 18) by some assay groups, certainly reflects a common inability of the test objects to simulate the highly specific receptor-mediated responses of the human body. The underestimation of the human toxicity of carbon tetrachloride (chemical 34) by most non-human assay groups may have alternative explanations. Specific enzymes to convert carbon tetrachloride to more-toxic intracellular metabolites could be lacking in the non-human cells, or the underprediction is sim-

ply caused by a too-low exposure in most systems of this highly volatile and insoluble chemical.

A look at the diverse new outliers from the correlations for the non-fish ecotoxicological tests reveals that the aquatic organisms, as well as the plant organs, seem to be generally more sensitive than human target organs, while the bacteria seem to be less sensitive. This could be due to the different systems used to expose test objects. In the aquatic and the plant organ systems, the test objects are directly exposed to the chemical, without pH buffering (except for the pollen tube test, method 58) or a potentially protective protein content of the medium. The general insensitivity of bacteria could be the result of a protective effect of the bacterial cell wall. Alternatively, it could be an effect of the short exposure time (5 minutes) in two of the bacterial tests (methods 49 and 50). This has been shown to generally decrease the sensitivity of tests (6–8). At the same time, this potential insensitivity is probably confounded by the very sensitive, non-lethal endpoint of the same two tests, i.e. inhibition of bioluminiscence.

As already discussed, a mechanistic interpretation of specific outliers in the correlations from the non-fish ecotoxicological tests is difficult. Only a few superficial comments can be made. It may be significant that as many as six of the nine outliers (overestimating human toxicity) from the correlation for the aquatic organisms are used as antiseptics or to control fungi, molluscs, worms or insects, i.e. phenol, potassium cyanide, copper (II) sulphate, mercury (II) chloride, hexachlorophene and pentachlorophenol (20). Copper (II) sulphate, mercury (II) chloride, hexachlorophene and pentachlorophenol are also strong human blood protein binders (9), which may lead to small toxic free fractions in human extracellular water, correctly predicted by the protein-free aquatic tests, but much less than the human serum concentration. Isolated and strong overestimation by the plant assays of human toxicity for the herbicide 2,4-dichlorophenoxyacetic acid may also be significant. Most of the other outliers in the plant correlation elude interpretation. However, for hexachlorophene and pentachlorophenol (chemicals 38 and 39), the overestimation by the plant assays may perhaps also result from the very high sensitivity to oxidative uncouplers of rapidly growing plant organs.

The general insensitivity of the bacterial tests adds further weight to the few overestimates of human toxicity. It may be significant that the three outliers, mercury (II) chloride, pentachlorophenol and chloramphenicol, all have have widespread uses as antibacterial agents. The underestimation of human toxicity for 11 compounds by the bacterial tests is difficult to interpret. Some of the outliers are probably due to a dissimilarity between the test systems and the human situation. For some chemicals, the bacterial results might also be nonsense average values, due to the many dissimilarities between exposure times, toxicity endpoints and test objects in this group (the photobacteria, the colibacteria and the halobacteria used in this study are very dissimilar bacterial species). Thus, the prediction of each system ought to be studied separately.

One clue to the outliers in the bacterial systems of five of the chemicals may be offered. Digoxin, verapamil hydrochloride, sodium fluoride, sodium oxalate and barium nitrate are the chemicals in the MEIC study which are known to affect the calcium and potassium balance of human cells at toxic concentrations. Digoxin increases intracellular calcium by effecting Na/K ATPase, verapamil hydrochloride inhibits transmembrane calcium flux, sodium fluoride decreases extracellular calcium and induces potassium flux from cells, sodium oxalate decreases extracellular calcium, and barium nitrate forces potassium into cells by affecting Na/K ATPase (9). Bacterial insensitivity to these chemicals could depend on the lower dependence on electrolyte levels, notably calcium levels, of bacterial cells compared with human and other vertebrate cells.

To conclude, the *in vitro* assays are all predictive of human toxicity for most chemicals, but prediction tends to decrease as the test systems are from species lower on the phylogenetic ladder. The best prediction of human toxicity in this study is provided by the human cell lines. To this statement must be added the fact that the study unfortunately did not include a good selection of primary cultures of human cells, which would be expected to give still better predictions, for instance, for digoxin. The surprisingly good correlation between the human toxicity and the *in vitro* results from phylogenetically

more-distant test objects have been noted before by some of the authors (21–23). According to one of these, the concept of basal cytotoxicity, originally defined as toxicity to common functions and structures of human cells (10, 11), could therefore be extended to the majority of eukaryotic cell types lacking the capacity to photosynthesise (22, 23).

It is thought by lay-people, as well as by some toxicologists, that there is a clear difference between human toxicology and ecotoxicology. Humans are believed to react to the toxicity of most chemicals at a higher level of organisation than do simpler organisms in the environment. The similarity of cytotoxicity, irrespective of species, found in the previous (6–9) and the present MEIC studies, and the good prediction of human acute toxicity by basal cytotoxicity for most chemicals found in this study, suggest that human toxicology is more intimately linked to ecotoxicology than is generally believed. It is likely that humans and other animals and plants are in the same situation, sharing the risks of most chemicals to about the same degree, set by the tolerance of the common traits of their cells.

Prediction of human lethal concentrations by human cell line assays

Several previous studies have tried to examine the relevance of *in vitro* cytotoxicity tests for animal and human acute toxicity by the comparison of results from cytotoxicity tests with LD50 values in rodents for a larger number (> 40) of non-selected chemicals (24–32). Often, oral LD50 values have been used, but some comparisons have involved intraperitoneal or intravenous LD50 values. Most often, g/l has been compared to g/kg, but sometimes moles/l has been compared to moles/kg. As a result, correlation coefficients (r values) between 0.46 and 0.80 have been obtained, indicating a positive, but not closely predictive correspondence of data. The variation in the results must depend on many factors. Three of these factors are: a) the rodent species compared; b) the route of administration compared; and c) whether g/l or molar comparisons were performed. The general impression from all the studies is that mouse LD50 gives a better correlation than rat LD50, that intravenous and intraperitoneal administration gives a better correlation than oral administration, and that molar comparisons result in better correlations than g/l comparisons.

Some of these studies have assessed the toxic actions of outlier chemicals (25–28 and 31). It was found that these outliers often had a known non-cellular animal toxicity (interaction with neurotransmission, etc.) or were biotransformed to more toxic metabolites by specific metabolic pathways not found in the cultured cells. Altogether, these results suggest a relevance of *in vitro* cytotoxicity for the animal acute toxicity for many compounds. Some relevance of the cellular tests for human acute toxicity can also be implied from these studies, due to the known similarity between rodent and human toxicity.

One of the authors of this paper interpreted the modest, but positive correlation between rodent LD50 values and *in vitro* cytotoxicities as an indication of a possible relevance of *in vitro* cytotoxicity for human toxicity for a large number of chemicals, obscured by: a) a distortion caused by the comparison of non-equivalent parameters (concentrations with doses); and b) a distortion caused by a comparison with rodent toxicity, which is certainly not identical to human toxicity (25, 26).

Therefore, human cell line cytotoxicities for 43 drugs in one assay (method 6 of the present study) were compared with calculated human acute lethal concentrations, including a mechanistic interpretation of the results (13). Some of the drugs not able to enter the CNS seemed to poison humans lethally at equivalent *in vitro* and *in vivo* concentrations, while most of the drugs with a free passage to the CNS led to a lethal CNS depression at around one tenth of the IC50 value. A few drugs with known non-cellular, specific actions poisoned humans by still smaller fractions of the IC50 value (< 1:100). The *in vivo* data were of poor quality in this study (13). For example, the human lethal concentrations were not authentic, but consisted of human lethal doses converted to concentrations by the use of distribution volumes (V_d). Thereby, the peaks of the many biphasic chemicals were excluded from the comparisons, which (as judged in retrospect) must have resulted in an overestimation of the blood–brain barrier compensation factor. Nevertheless, the results led to the basal cytotoxicity concept, i.e. that a clear majority of non-selected chemicals are acutely lethal

to humans through their basal cytotoxicity, which can be measured *in vitro* in cell lines (10, 11). One of the main reasons for initiating the MEIC programme was to see whether these results could be repeated in a larger, more refined study.

Since then, many studies have compared *in vitro* toxicity results from tests on the MEIC chemicals in separate systems with rodent LD50 values (33–38) or human lethal dose values (33, 35). The correlations found have been good, but not closely predictive, as for the previous similar comparisons of non-MEIC compounds. The best correlation ($r = 0.88$) was obtained between *in vitro* IC50 values and rat intravenous LD50 values (37). One study tried to decrease the potential distortion of the concentration/dose comparison by introducing an algorithm based on physicochemical data (38).

A few preliminary studies by MEIC participants have compared *in vitro* results from individual MEIC assays (also submitted to MEIC) directly with human lethal blood concentrations (21, 39–42). The set of human lethal concentrations was specific for each study and, as a rule, were compiled from only a few sources. The correlative results were generally better than the results from the previously discussed concentration/dose comparisons. Thus, methods 1, 10, 24 and 58 of the present study, used to test all 50 chemicals (missing values not counted), had correlation coefficients (r values) of 0.80, 0.75, 0.48 and 0.75, respectively (39, 40). Furthermore, it was shown that rodent lethal concentrations, derived algorithmically from rodent LD50 values, did not give a significantly better prediction of the human lethal concentrations (39, 40). The correlations obtained were similar to the correlations found in the present study between *in vitro* toxicity of separate tests and clinical lethal concentrations (Table II). No attempts were made in these preliminary studies to systematically discuss outlier chemicals in mechanistic terms.

Similarly to the goals of previous studies, the aim of the present study was limited to examining the relevance of the most predictive group of *in vitro* assays. Lethal concentrations over defined exposure times to cells *in vitro* (IC50 values) were compared with human lethal serum concentrations at defined exposure times, thought to represent concentrations of the chemicals surrounding cells in the extracellular fluid of the target organs. As mentioned earlier, the actual comparability of IC50 values and human lethal concentration values is connected with the analogy of the systems compared.

The typical 24-hour human cell line assay is a static, one-compartment system, in which medium containing 5–10% serum holds less than 0.1% cells/water volume (methods 1–10 use media with an average of 6.5% serum; Table II of Part IV [8]). The human body is a dynamic, essentially two-compartmental system (3 litres of serum and 12 litres of extracellular water), in which the extracellular water/blood containing 20% serum holds about 50% cells/water volume. Of these incongruencies between the systems, the differential cell density must be the most important, followed by the different extracellular protein contents (25).

The lower cell density in the *in vitro* system leads to equivalent initial concentrations and post-distribution concentrations, irrespective of the degree of accumulation of the chemicals. However, such chemicals in the human body may have lower post-distribution concentrations when compared to pre-distribution concentrations. Thus, only post-distribution, steady-state human lethal concentrations are wholly comparable to IC50 values. If pre-distribution lethal concentrations are compared, they may be too high. This potential incongruency of the compared concentrations could not be accounted for in this study, due to the shortage of raw data on the time for full equilibrium between extracellular water and target cells *in vivo*.

The effects of the variation in protein content between systems can be estimated. In the 20% serum human system, the serum content reduces the free extracellular water fraction of chemicals in proportion to their serum binding. Thus, the human lethal concentration for a chemical with 90% protein binding overestimates the free fraction by a factor of ten. In the typical 6.5% serum cell culture medium, the free fraction is also reduced by the serum content, but only by a factor of about 1.7. If the IC50 value of a chemical with a $\geq 90\%$ blood protein binding is compared with the human lethal concentration, the IC50 value ought to be increased by a factor of $10/1.7 = 6$, or more, to be truly comparable in terms of equal free fractions reaching the target cells. The corresponding

factor for ≤ 75% or less protein binding is only ≤ 3.3. In-depth calculations will be presented in a future paper.

The data on human blood protein binding at low-dosage are very incomplete for the 50 MEIC chemicals. Reliable binding data for lethal dosages are almost absent (Table V of Part V [9]). Therefore, the variation of IC50 values and lethal concentration values could not be systematically considered in the present study, but will be analysed in a forthcoming paper for chemicals with reasonably reliable protein binding data. The IC50/lethal concentration comparisons for a few listed chemicals (see Methods and Results), may have been adversely affected by their high protein binding but, on the whole, this confounding factor is not larger than other errors in the raw data.

Similarities and dissimilarities between the *in vitro* IC50 values and the human lethal concentration values have been analysed with a sequential correlative/mechanistic approach, adapted to achieve two aims. The approach had to be rigid and follow predetermined rules (1, 2), to convincingly test the basal cytotoxicity hypotheses, while it also had to be versatile enough not to miss any opportunities to reveal the true relevance of the assays.

The main results are found in Figures 8, 10 and 11, while Figures 4–7, 9, 12, and Table V present only the results of successive phases of the analysis. The aim of the following discussion is not to comment on details of each step. Instead, some crucial steps will be further explained before the results are discussed and conclusions from them are drawn.

Figures 4–6 show the basis for the correlative/mechanistic study. They also serve to connect the study with the previous studies on the prediction of human toxicity/rodent LD50 values and by the 61 MEIC assays. They are not directly comparable with the rodent LD50 prediction of human toxic doses (Figures 2 and 3), due to the comparison of concentrations. Some of the lethal doses from the literature may not be directly connected with the lethal concentrations found in the same sources. However, they can be compared, in a general sense, with rodent predictions, since both sets of correlations represent predictions of human toxicity. When compared with the results of the rodent LD50 correlations (R^2 = 0.61–0.65), the plots show a better prediction of human toxicity by the average 24-hour IC50 values (R^2 = 0.67–0.74), irrespective of the set of human lethal concentrations used in the comparison. This is a surprising result, which can be better understood in light of the results of the subsequent correlative/mechanistic study.

The decision to introduce a factor of ten as a hypothetical compensation for an extra sensitivity of the brain, was a part of the predetermined analysis. It was not possible, at this early stage of the analysis, to choose a more optimal factor, due to lack of knowledge of the suboptimal correlation for the slow-acting chemicals and the mechanistic proof of some outlier chemicals in the CNS group (Figure 6b).

The decision to compare average 48-hour IC50 values with 48-hour values from approximative LC50 curves over time was a part of the versatile analysis. The decision is typical of hard modelling, which always tries to select the comparisons on a rational basis (1). The positive results of this step were encouraging. At the beginning of the analysis, it was thought that the correlations of Figure 7 and Table V represented the best available prediction.

The attempt to perform soft modelling of the total results to get a better idea of the significance of the minor outliers (Figure 12) was again a versatile part of the analysis. The procedure was not a fundamental part of the study, but rather shed some light on a specific problem. The plot suggested that the factor of ten used to compensate some IC50 values for the supposed CNS sensitivity was too large. At the same time, the tentative new factor of 3.2 was determined in a less exact way than the previous factor. The correlation, as well as the regression line, of the plot cannot be considered as bona fide, since it was made up of a mixture of 24-hour and 48-hour IC50 values, as well as of peak concentrations and 48-hour human lethal blood concentrations. The final adjustment of the blood–brain barrier conversion factor belongs to the final battery optimisation in the MEIC programme. This optimisation must be based on a still better discrimination between relevant and irrelevant correlations for individual chemicals, which can only be reached by the comparisons for each chemical to be made in a forthcoming paper.

The correlative results presented in Figures 8, 10 and 11, together with the known traits of the actions and kinetics of the chemicals, indicate a relevance for human lethal action (peaks or 48 hours) of human cell line cytotoxicity (24 hours or 48 hours) for most of the 50 chemicals in the MEIC study. A few of the chemicals have an organ-specific or organisational (1, 11, 12) human toxic mechanism which cannot be tested in cell cultures, i.e. digoxin, malathion, nicotine and atropine sulphate. A few chemicals (potassium cyanide, paracetamol, paraquat, ethylene glycol and methanol) have important lethal effects which have probably not been detected by the human cell line cultures. The good correlation for potassium cyanide was probably due to an incorrect lethal concentration. The good correlation for the other four chemicals may be due to a fortuitous similarity between the approximate LC50 curve for the CNS-depressive effect and the approximate LC50 curve for the second, non-CNS lethal, effect.

The overview correlations of groups of chemicals made in this first evaluation study, as well as: a) the inexactness of some of the human base data of the study; b) the uncertainity of the proper blood–brain barrier conversion factor; c) the uncertain information on the blood–brain barrier passage for a few chemicals (including the two confounding chemicals, paracetamol and paraquat); and d) the potential variation of IC50 values and lethal concentrations for some chemicals with high protein binding and high distribution volumes, make it difficult to tell the exact number of the 50 chemicals with an irrelevant human cell line toxicity. The number is possibly below eight, and certainly below ten. The picture of borderline outliers will be made more precise by the forthcoming detailed studies.

The general, as well as some specific, results of the study confirm and clarify most of the results of previous studies (21, 24–42), notably, the study comparing 43 other drugs by using different methodology (13). The hypotheses on basal cytotoxicity should be considered proven by the present results, together with results of previous papers in this series (6–9). In fact, the present results surpass expectations at the time of the proposal of the basal cytotoxicity concept (10, 13).

One conclusion is that the high relevance of *in vitro* cytotoxicity tests demonstrated with human cells would ultimately make it possible to provide highly predictable batteries of such tests and supplementary toxicokinetic tests (1, 10, 11). A core of tests for these batteries, based on the best combination of the 61 assays contributed to this study, will be provided in future papers of this series. Note that the demonstrated relevance of *in vitro* results for a high percentage of chemicals does not mean that a currently available battery is able to correctly test a corresponding number of chemicals. New tests and test strategies must be developed before batteries can make full use of the demonstrated relevance. Therefore, the EDIT programme (Evaluation-guided Development of new *In vitro* toxicity and toxicokinetic Tests) will be initiated (43–45).

Another conclusion is that the high relevance of human cell tests demonstrated here will permit *in vitro* studies in simple systems of the actual toxic mechanisms for most chemicals, which would lead to rapid progress in toxicology. It is very difficult to reveal specific mechanistic knowledge of acute lethal toxicity through clinical studies or animal experimentation. It is much easier to perform primary studies in cell cultures, and to confirm these studies at a later stage by clinical or animal studies. At the moment, the actual mechanisms of the acute lethal toxicity for most chemicals are unknown, as judged from the MEIC human toxicity database (9). If the mechanisms can be revealed by primary cell studies of most chemicals, victims of poisonings will be treated more rationally in the future. Also, the toxicological risk assessment process will benefit from an improved knowledge of mechanisms. Therefore, the new approaches in research on such mechanisms should be welcomed.

A final conclusion concerns the interpretation of the lethal symptoms of victims to acute chemical poisoning. The results clearly indicate that non-specific CNS excitation/ depression is an obligatory reaction of humans to cytotoxic concentrations of all chemicals that easily cross the blood–brain barrier. Concomitant action on heart and blood vessels might be a parallel direct action of the cytotoxic concentrations to two other organs of short-term (minutes–1 hour) vital importance. Causes of death related to non-CNS organs are most often due to cytotoxic concentrations of chemi-

cals unable to cross the blood–brain barrier very easily. Some of the latter chemicals kill humans by a short, relatively specific and varied action by the peak concentration (like most CNS-depressive chemicals). Others kill by a slow action of the cytotoxic concentration on many non-CNS organs, thereby inducing morphological lesions in the organs. Hence, the main features of the most typical human poisoning syndromes seem to be a function of the onset and duration of action, as well as the distribution to CNS, or, alternatively, other organs, of basal cytotoxic concentrations, irrespective of the exact mechanism for this cytotoxicity. In this paper, only the CNS/non-CNS distribution of cytotoxicity has been discussed. In the planned Part VIII paper, target-organ toxicity will also be related to distribution of cytotoxic chemicals among non-CNS organs, to try to explain concomitant, minor features of the different poisoning syndromes.

Acknowledgements

The work by the MEIC management was made possible by the financial support of the Swedish National Board for Technical Development (Stockholm, Sweden), the Swedish National Board for Laboratory Animals (Stockholm, Sweden), the Swedish Fund for Research without Animal Experiments (Stockholm, Sweden), the American Fund for Alternatives to Animal Research (AFAAR; New York, USA), the New England Anti-Vivisection Society (NEAVS; Boston, MA, USA), the Marchig Animal Welfare Trust (Geneva, Switzerland), the Society of Animal Friends (Helsingborg, Sweden), the Juliana von Wendt Animal Protection Fund (Helsinki, Finland), the Body Shop Foundation (Littlehampton, UK), Estée Lauder Companies (New York, USA), Sandoz Pharma AG (Basel, Switzerland), CIBA-GEIGY AG (Basel, Switzerland), the Wellcome Foundation Ltd (London, UK), GAMBRO AB (Lund, Sweden), Chevron Corporation (Richmond, CA, USA), Sun Chemical AS (Köge, Denmark) and N.V. Organon (Oss, The Netherlands). We also wish to express our gratitude for the financial support from numerous sources to the practical testing in each of our laboratories.

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Authors' Addresses

[1]*CTLU, Pavals, När, 620 13 Stånga, Sweden;* [2]*Department of Natural Sciences, York College, City University of New York, 94-20 Guy R. Brewer Boulevard, Jamaica, NY 11451, USA;* [3]*Environmental Toxicology, Centro de Investigacion en Sanidad Animal, Valdeolmos, 28130 Madrid, Spain;* [4]*School of Biomedical Sciences, Queen´s Medical Centre, Nottingham NG7 2UH, UK;* [5]*Instituut voor Hygiene en Epidemiologie, Juliette Wytsmanstraat 14, 1050 Brussels, Belgium;* [6]*Institute of General Pathology, Via L.B. Alberti 2, 16132 Genova, Italy;* [7]*Department of Genetics, University of Lund, Sölvegatan 29, 223 62 Lund, Sweden;* [8]*Departament de Farmacologia y Toxicologia, Facultad de Medicina, Universidad Autonoma de Nuevo Leon, Apartado Postal No. 146, Col. del Valle, Nuevo Leon, Mexico;* [9]*Cultivos Celulares, Centro Investigacion, Hospital La Fe, Av. Campanar 21, 46009 Valencia, Spain;* [10]*Institut für Toxicologie, Zelltoxikologie, Christian-Albrechts-Universität, Weimarer Strasse 8, Haus 3, 24106 Kiel, Germany;* [11]*MD Laboratories, B.P. 30, 68870 Bartenheim, France;* [12]*Department of Biology, Åbo Akademi University, Biocity, Artillerigatan 6, 20520 Åbo, Finland;* [13]*Laboratory for Molecular Genetics, Institute of Chemical Physics & Biophysics, Estonian Academy of Sciences, Akadeemia tee 23, EE-0026 Tallinn, Estonia;* [14]*Institute of Life Science and Chemistry, P.O. Box 260, 4000 Roskilde, Denmark;* [15]*Institut für Allgemeine Botanik, Universität Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany;* [16]*Environmental Health Sciences Division, National Institute of Environmental Studies (NIES), 16-Z Onogawa, Tsukuba, Ibaraki 305, Japan;* [17]*Department of Biochemistry and Biotechnology, University of Kuopio, P.O. Box 1627, 702 11 Kuopio, Finland;* [18]*Department of Animal Physiology, University of Lund, Helgonavägen 3D, 223 62 Lund, Sweden;* [19]*CETA - Centre for Ethical Treatment of Animals, 39-3-23, Volzsky Bulvar, 109462 Moscow, Russia;* [20]*RIKEN Cell Bank, Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba City, Ibaraki 305, Japan;* [21]*Laboratory for Biological Research in Aquatic Pollution, University of Ghent, J. Plateaustraat 22, 9000 Ghent, Belgium;* [22]*Pharmacia & Upjohn, 112 87 Stockholm, Sweden;* [23]*Biomedical Defence Section, Defence Research Establishment Suffield–DRES, Box 4000, Medicine Hat, Alberta T1A 8K6, Canada;* [24]*Vitro-Bio, 11, Allée de la Source, 63340 Le Breuil-Sur-Couze, France;* [25]*Umweltforschungs-zentrum, Leipzig-Halle GmbH, Sektion für Chemische Ökotoxikologie, PF-2, 04301 Leipzig, Germany;* [26]*Department of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Roma, Italy;* [27]*Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano, Kanagawa 257, Japan;* [28]*Universitá di Ancona, Clinica del Lavoro, Ospedale Regionale, 60020 Ancona, Italy;* [29]*Istituto Tecnologie Biomediche, Consiglio Nationale delle Recherche, Via G.B. Morgagni 30/E, 00161 Roma, Italy.*